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**THE FERMENTATION ORGANISMS OF**  
**CALIFORNIA GRAPES**

BY  
W. V. CRUESS

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# THE FERMENTATION ORGANISMS OF CALIFORNIA GRAPES<sup>1</sup>

BY

W. V. CRUESS

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<sup>1</sup> The writer wishes to thank Professor F. T. Bioletti for his helpful suggestions for carrying out these investigations and preparation of the manuscript.

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## INTRODUCTION

The manufacture of wine, grape-juice, and raisins and the shipping of grapes depend very largely upon the control of micro-organisms, particularly of those occurring naturally upon the fruit. This economic fact and the scientific interest of the subject led to a study of the micro-organisms occurring on the grapes of California. The investigation was qualitative and quantitative, covering the effect on type and number of micro-organisms of (*a*) locality, (*b*) degree of ripeness, (*c*) shipment from vineyard to winery. It included studies of (*d*) the micro-organisms normally found on grapes as received at the winery, (*e*) their control in fermentation, and (*f*) their morphological and physiological characteristics.

## I. GENERAL DISCUSSION OF GRAPE MICRO-ORGANISMS

Several groups of micro-organisms are normally found on grapes. These may be classified under the general terms: (1) Budding fungi, including molds, true yeasts which form spores, and pseudo-yeasts, which do not form spores; and (2) fission fungi, including bacteria (non-motile rods), bacilli (motile rods), the various forms of *Coccaceae*, and the fission yeasts.

Representatives of all the above groups except the bacilli, *Coccaceae*, and fission yeasts were found in California grapes. Most attention has been given to the fermentation organisms: *i.e.*, yeasts. The molds and bacteria have in most cases been merely listed.

The more important forms of organisms occurring on grapes are the following:

## MOLDS

*Penicillium*.—This group includes a large number of varieties, which are the most widely distributed and common of all the molds. They are characterized by the method of formation and grouping of conidia shown in a typical manner in figure 1.

The most common mold of this group met with on grapes is *Penicillium glaucum* or *Penicillium expansum*. It is the common green mold and is the cause of moldy flavors in grapes, barrels, etc.

During the first stages of growth it appears as a cottony, white mass of mycelial threads. These white threads soon develop fructifications bearing large numbers of conidia that are green in young cultures and brown in old. These give a powdery appearance to the culture. The individual conidiophores are branching and present a broomlike appearance under the microscope.

It does not carry on alcoholic fermentation, but may destroy sugar by oxidation to  $\text{CO}_2$  and  $\text{H}_2\text{O}$  or the formation of penicillic acid.

*Aspergillus niger*.—This is a black mold of very common occurrence on California grapes (see fig. 1). On grapes and must it forms a white mycelium from which spring short rods bearing aggregations of black cells. These conidiophores are not branching, thus differing from penicillium. These groups of cells are easily discernible with the unaided eye. On the grapes of some districts the spores or conidia may be broken away from the main growth of the mold during picking and rise as a black dust. At present it is not held to be very harmful in wine making.

*Oidium or Powdery Mildew of the Vine*.—This fungus is of more concern to the grape grower than it is to the wine maker and will be found fully described in Bulletin 186<sup>2</sup> of the University of California Agricultural Experiment Station (see fig. 1). This mold may prevent the grapes reaching maturity or may cause them to crack and thus to be liable to attack by penicillium. In both cases, the grapes affected may become unfit for wine making. The microscopical appearance of the summer form of the powdery mildew is shown in figure 1. It occurs most commonly as a downy white growth on the leaves, canes, and grapes during moist or foggy weather and is most prevalent during early summer. It forms winter spores or perithecia on the canes, in which form the organism lives through the winter.

<sup>2</sup>Bioletti, F. T. (*Oidium or Powdery Mildew of the Vine*), Univ. Calif. Exp. Sta., Bull. 186, pp. 317-327, 1907.

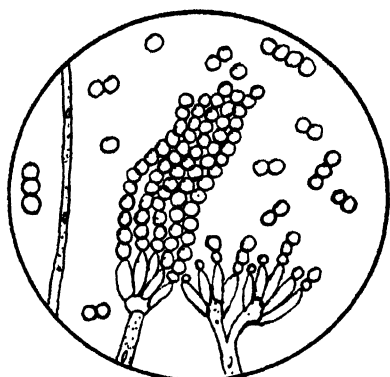
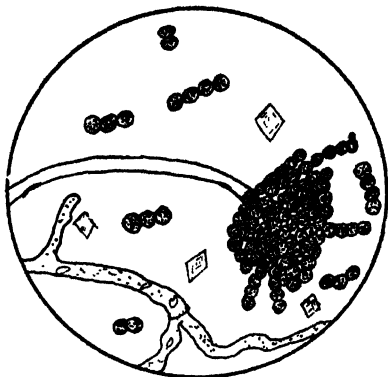
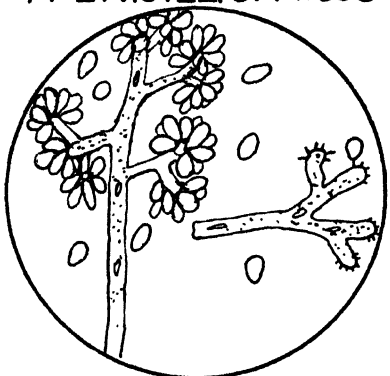
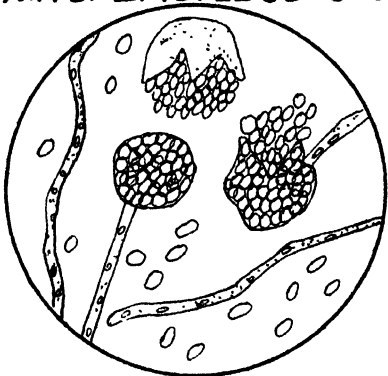
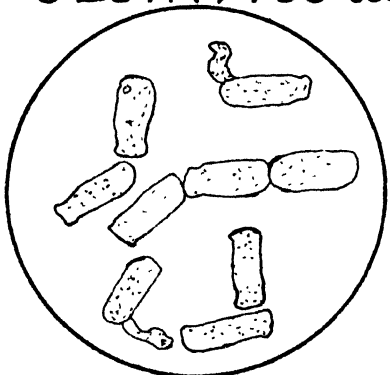
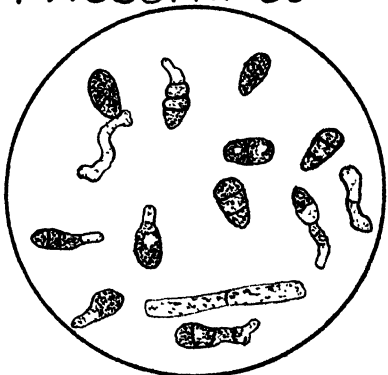
1. *PENICILLIUM*  $\times 800$ .2. *ASPERGILLUS*  $\times 800$ .3. *BOTRYTIS*  $\times 800$ .4. *MUCOR*  $\times 150$ .5. *OIDIUM*  $\times 800$ .6. *BROWN MOLD*  $\times 800$ .

Figure 1

Molds from California grapes:

1. *Penicillium* variety from California grapes, magnified 800 diameters.
2. *Aspergillus* variety from California grapes;  $\times 800$ .
3. *Botrytis cinerea* from California grapes;  $\times 800$ .
4. *Mucor* variety from California grapes;  $\times 150$ .
5. *Oidium* or Powdery Mildew from California vine, summer form;  $\times 800$ .
6. Brown mold, parasitic fungus from California grapes;  $\times 800$ . Species and variety not determined.



*Botrytis cinerea*.—This mold is a parasite or facultative saprophyte occurring on grapes in moist climates (see fig. 1). It rarely develops on California grapes during the wine making season but is often found on grapes left on the vine during the winter. It appears as a gray, matted growth on the surface of the grapes. The individual fructifications may be seen with the naked eye on short upright conidiophores. Under the microscope, the conidia may be seen in grapelike clusters.

The mold did not grow readily in culture media, but will develop profusely on infected grapes in a moist jar.

In Europe, the *Botrytis* is considered beneficial in the Sauternes district, because it causes a concentration of the sugar content of the grapes by favoring evaporation of the moisture. It produces also an oxydase that acts vigorously upon the color of the grapes or wine.

*Mucor*.—*Mucor* mold will nearly always be found in an examination of California grapes (see fig. 1). Since it develops very slowly in comparison with the commoner molds and yeasts found on grapes it is not probable that it does very much harm in wine making.

In pure cultures, it produces a gray filamentous mass of mycelial threads, from which upright rods are given off, bearing at their uppermost ends spherical sporangia filled with large numbers of spores. These sporangia are easily seen with the unaided eye. Most of the *mucor* molds are capable of forming yeastlike cells and carrying on a feeble alcoholic fermentation in sugary liquids.

*Monilia*.—The *monilia* molds and especially *Monilia candida* occur very commonly on fruits of all kinds (see fig. 2). Most of the grapes examined in 1912 bore considerable numbers of the cells of this organism.

In young cultures, it forms colorless yeastlike cells and gives a feeble fermentation. The fermentation is followed by a moldlike growth on the surface of the grape must, etc., which in old cultures becomes olive green in spots.

## TRUE YEASTS

*Culture Yeasts*.—(1) *Saccharomyces ellipsoideus* (True Wine Yeast). In general, the *ellipsoideus* yeasts are characterized by rapid growth in grape must with the production of a strong fermentation, yielding 10% to 16% of alcohol. They differ from the beer yeasts, *S. cerevisiae*, principally in their higher alcohol-forming power and

in the flavor of the fermented liquids, the wine yeast giving a vinous flavor to fermented liquids and the beer yeast a beer flavor. In grape must, the *S. ellipsoideus* forms a cloudy growth and a pasty or granular sediment in the bottom of the container during the main fermentation. After fermentation is complete the suspended cells settle

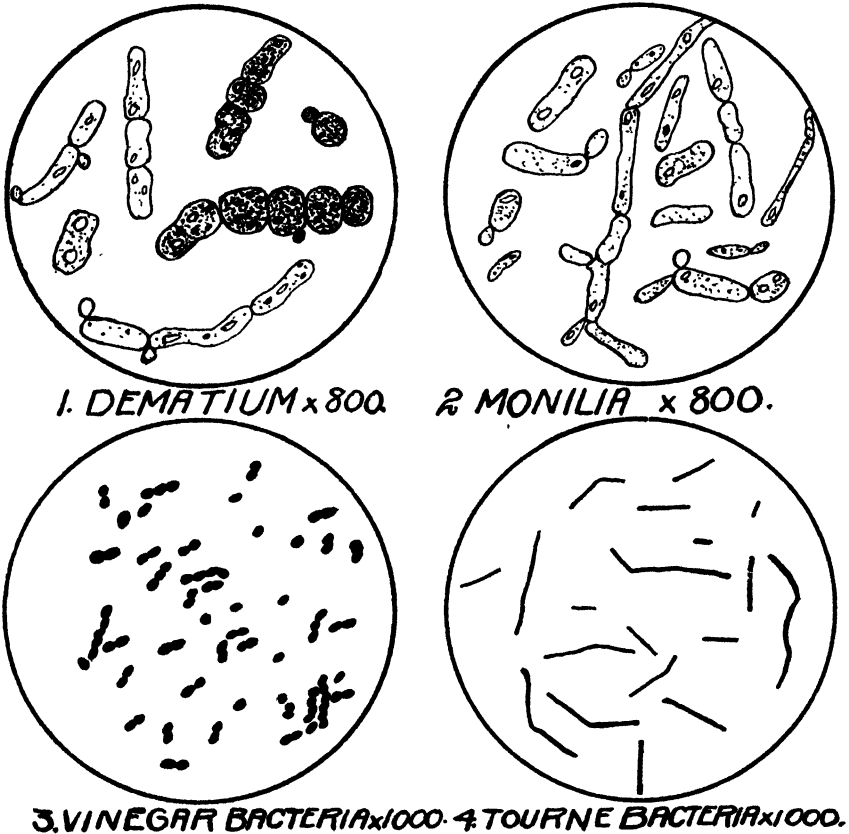


Figure 2

Molds and yeasts from California grapes:

1. Dematium variety from California grapes;  $\times 800$ .
2. Monilia variety from California grapes;  $\times 800$ .
3. Vinegar bacteria from California grapes;  $\times 1000$ .
4. Tourne bacteria from California wine;  $\times 1000$ .

out, giving a clear liquid and compact sediment. Under suitable conditions spores are formed. The usual shape of the cells is shortly ellipsoidal, although this will vary from spherical to elongate, with the different varieties. The shape of the spores is spherical. The appearance of several specimens of *S. ellipsoideus* from California grapes and of cells containing spores will be found in figures 5 and 6.

The main part of all wine fermentations is carried on by this yeast. There are a great many different varieties in this group and these vary considerably in their suitability for wine-making purposes. Modern methods of wine making aim to make use of the most desirable varieties.

2. *Saccharomyces cerevisiae* (B  r Yeast).—It is possible that these yeasts occur on grapes to a more or less limited extent, depending upon the proximity to vineyards of breweries or distilleries using these yeasts. No reference has been found in the literature describing their occurrence on grapes and none have been found on California grapes. There is probably little likelihood of their being present on grapes in sufficient numbers to influence in any way the fermentations. This yeast is worthy of mention in connection with wine making, however, because it has been used in the past to start wine fermentations. Where brewery yeast is so used, it produces a wine of beerlike flavor and low alcohol content and with an excess of unfermented sugar. Such a wine is not palatable and is very liable to be attacked by bacteria and to be lost through bacterial decomposition.

3. *Saccharomyces malei*.—This yeast occurs on apples and is similar to *S. ellipsoideus*, but usually forms less alcohol. It was not found on any samples of grapes examined, but probably occurs occasionally on grapes.

*Wild Yeasts*.—(1) *Saccharomyces pastorianus*.—This group of yeasts is characterized by its elongate or sausage-shaped appearance and its ability to form spores. Members of this group of yeasts have been found on two samples of California grapes. They form small amounts of alcohol in grape must and at the same time usually produce undesirable flavors and odors—usually a bitter flavor. The microscopical appearance of a culture of *S. pastorianus* yeast from California grapes is shown in figures 3 and 6.

2. *Saccharomyces anomalus* (*Willia Yeasts*).—The yeasts of this group are characterized by the production of hat-shaped spores. The appearance of such spores is shown in figure 7. The members of this group grow rapidly in must and form a wrinkled film. They carry on a weak fermentation with the production of small amounts of alcohol. Liquids fermented by these yeasts are high in aromatic compounds of various sorts and for this reason their use has been suggested as a means of flavoring various fermented beverages. Cells from a culture isolated from California grapes are shown magnified 1000 diameters in figures 3 and 6.

3. *Saccharomyces ludwigii*.—This yeast has been found on grapes grown in Europe. In microscopical appearance it resembles the *S. apiculatus* yeast, but is much larger. It was not found on California grapes.

4. *Saccharomyces marxianus*.—This yeast has been reported as being present on grapes. It is recognized by its kidney-shaped spores. It was not found on the samples of California grapes examined.

#### PSEUDO-YEASTS

*Apiculatus* Yeast.—The *apiculatus* yeast (*Saccharomyces apiculatus*, *Hansenia apiculata*) is recognized by the peculiarly pointed appearance of many of its cells (fig. 3). According to most authorities it does not form spores and so is placed in the group of pseudo-yeasts. According to Lindner, however, it forms spores in drop cultures, one spore per cell. It carries on a feeble bottom fermentation in grape must but does not have the power to ferment saccharose, maltose, or lactose. In must it gives from 0% to 6% alcohol and at the same time produces fruity flavors and odors. It settles more slowly and less completely than *S. ellipsoideus*. *Apiculatus* yeast probably does more harm in wine making than all other varieties of wild yeasts combined, because of its large numbers on grapes and its very rapid development after the grapes are crushed. It develops so rapidly that the first stages of most natural wine fermentations are carried on by this yeast and the preliminary fermentation of grapes is often spoken of as the "apiculate" stage. During this preliminary fermentation it produces undesirable flavors and aromas, destroys yeast food that should have gone to the true wine yeast, forms compounds deleterious to the vigorous development of the true wine yeast and gives a great many cells of low specific gravity that settle out slowly after the main fermentation is over. After the *S. ellipsoideus* yeast has formed 8 to 10 per cent alcohol the *Apiculatus* yeast is killed and will not be found in the living state in the finished wine.

*Mycoderma* Types.—The *Mycoderma* forms are known to all wine makers as "wine flowers" (fig. 4). They are present in considerable numbers on uncrushed grapes and have been found on nearly all samples of California grapes so far examined. In pure cultures in liquids, they appear as white films, usually more or less deeply wrinkled. From time to time portions of the pellicle are detached and fall to the bottom giving in time a large amount of sediment. A

feeble fermentation is carried on at the surface of the liquid, provided that it contains fermentable sugar. Under the microscope the yeast appears as cells of irregular shape and size, the usual form, however, being elongate or sausage-shaped. The cells tend to adhere together in groups and chains. It is thought that the cells are maintained at the surface of the liquid by means of small bubbles of air occluded between them.

In pure cultures in grape must, the *Mycoderma* yeasts are capable of destroying varying amounts of sugar with the production of small amounts of alcohol, in most cases, and obnoxious flavors and aromas. It is probable that in many natural wine fermentations they are more or less active at the beginning of the fermentation. They undoubtedly cause trouble in tanks of wine that are not kept well filled during storage. They are aerobic.

*Torula Yeasts.*—Under the heading of *Torula* are placed most of the yeasts that do not fit into other groups. Consequently, this name covers yeasts of wide variation in properties. As a group they do not form spores; they form small amounts of alcohol, do not normally develop as a film growth, and the form considered as typical is spherical, although there is considerable variation from the typical form. They are found on grapes fairly commonly, though less abundantly than *S. apiculatus* and *Mycoderma*. Two forms from California grapes are shown in figure 3.

## II. PROPERTIES OF MOLDS AND BACTERIA FROM CALIFORNIA GRAPES

### SOURCES OF CULTURES

In 1911 micro-organisms were isolated from grapes received at the winery of J. E. Colton, Martinez, California, and from grapes picked at the University Farm, Davis. Samples were obtained in 1912 from Fresno and El Centro. The properties of these micro-organisms were studied more or less in detail. Most attention was paid to the organisms and characteristics that were of importance in wine making.

*Method of Separation.*—The materials used in the separation of the various types of organisms present on the grapes were sterile grape must agar, sterile grape must, petri dishes, and a small platinum inoculating rod. The grape must agar was made by dissolving 20 grams of agar agar in 1000 c.c. of boiling water to which was added 60 c.c. of grape must after the agar had dissolved. It is necessary

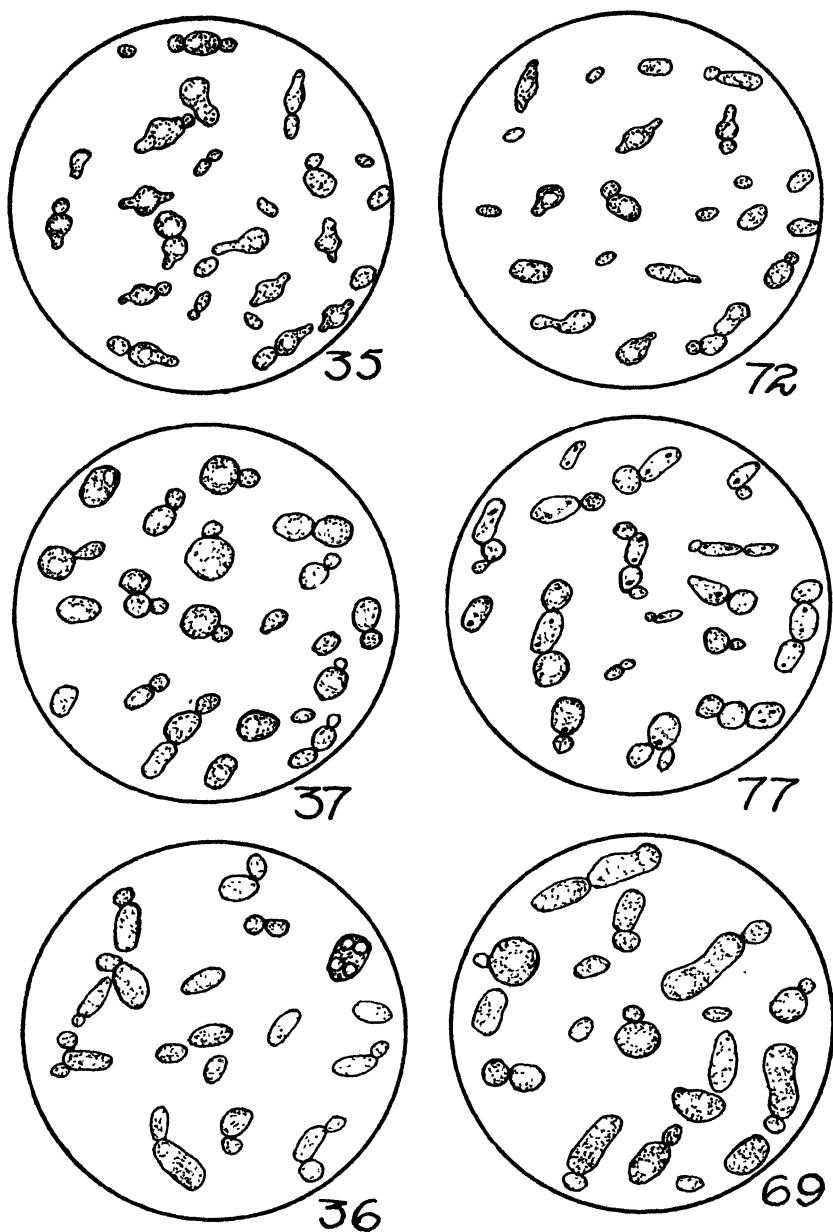


Figure 3

Yeasts from California grapes magnified 1000 diameters:

- 35. *S. apiculatus* from Davis grapes.
- 72. *S. apiculatus* from Acampo grapes.
- 37. *Torula* from Davis grapes.
- 77. *Torula* from Contra Costa County grapes.
- 36. *S. pastorianus* from Davis grapes.
- 69. *Willia* species from Acampo grapes.

to use this high dilution of must in the agar in order that the agar will not be hydrolyzed by the acid of the must. At this dilution the agar contains about .05 per cent acid as tartaric. This should permit the growth of all forms. The hot agar solution was filtered through cotton wool and filled in 10 c.c. portions into test tubes plugged with cotton wool. It was sterilized at 15 pounds pressure in an autoclave. The grape must was clarified by boiling with the white of egg and filtration. The clear must was filled into plugged test tubes and sterilized at 100° C. The Petri dishes were sterilized in packages of three by dry heat. The type of dish used is shown in figure 3.

Samples of the grapes received at the winery were taken from the center of the boxes and crushed into sterile containers. A small amount of the must was in each case transferred by a sterile platinum wire to a tube of melted agar kept at 40° C to 45° C, and further dilutions were made by transfer to other tubes of agar.

The organisms were separated by growth on agar must. They were purified by replating on the same medium. Subcultures were then made in sterile must, on agar slants and permanent stock cultures were made in sterile 10 per cent cane sugar in Freudenreich flasks.

The pure cultures obtained in this way are discussed in groups, the members of each group having certain characteristics in common. No attempt has been made so far to study the molds and bacteria in detail and for this reason they have simply been listed with a few words of explanation.

### MOLDS

*Penicillium Species* (probably *Penicillium expansum*).—Found on nearly all samples examined; produces fructifications bearing numerous round conidia as shown in figure 1; forms sclerotia, resistant vegetative forms, in old cultures; no fermentation in must, but grows vigorously in all culture media tested; giving a characteristic moldy odor.

*Penicillium Species of Olive Green Color*.—Forms ellipsoidal conidia; very common on California grapes and also found in samples of pasteurized unfermented grape juice that had molded after pasteurization.

*Aspergillus*.—Two forms: (a) produces small, smooth, black conidia; (b) produces large black conidia with spikelike projections. The appearance of the one yielding the smooth conidia is shown in figure 1.

*Botrytis* (probably *B. cinerea*).—Found on many samples of grapes

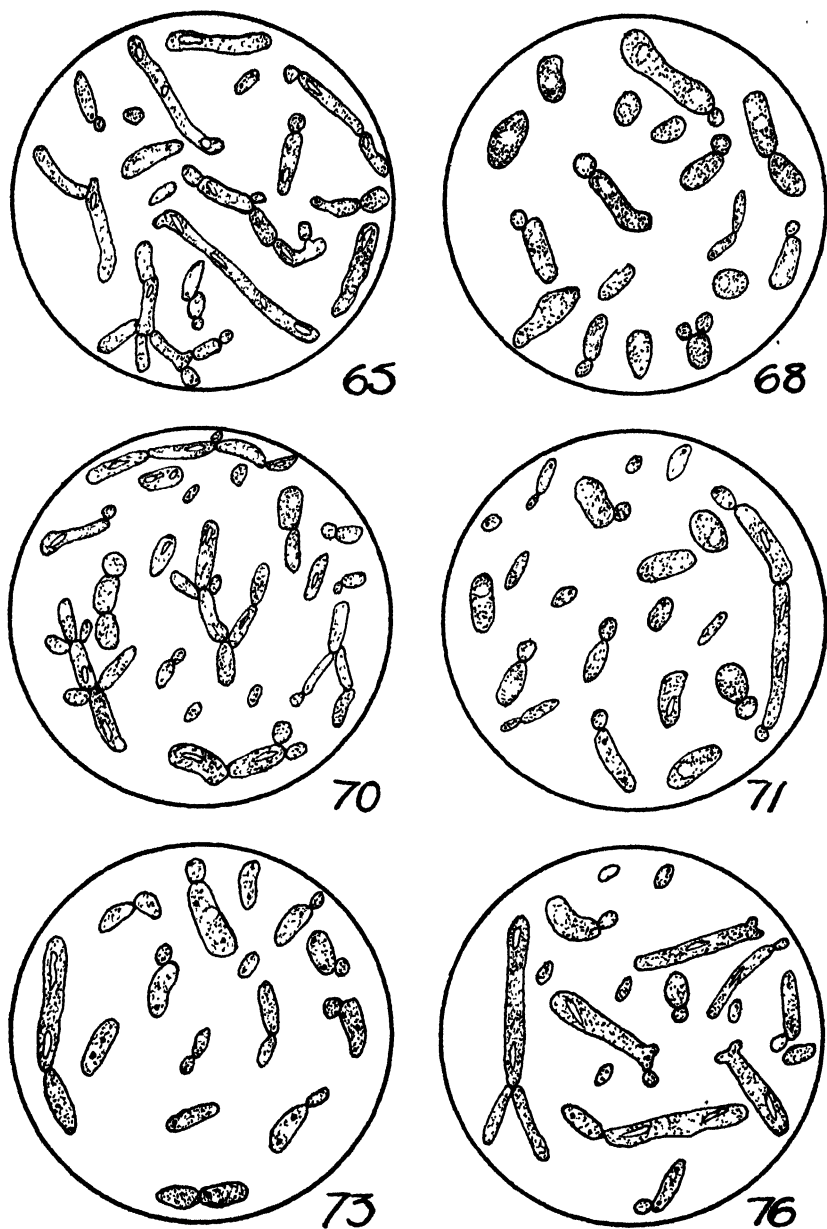


Figure 4

Yeasts from California grapes:

- 65. *Mycoderma* yeast from Acampo grapes.
- 68. *Mycoderma* yeast from Acampo grapes.
- 70. *Mycoderma* yeast from Acampo grapes.
- 71. *Mycoderma* yeast from Contra Costa Grapes.
- 73. *Mycoderma* yeast from Acampo grapes.
- 76. *Mycoderma* yeast from Acampo grapes.



after the early rains of 1912. It gives the characteristic *Botrytis* growth on the grapes but is very reluctant to grow on any of the ordinary culture media, the best artificial medium found so far being grape must gelatin made up by dissolving gelatin in undiluted grape must. The appearance of this mold from grapes grown at the vineyard of J. Swett and Son, Martinez, is shown in figure 1.

*Mucor*.—Color, gray; spores, shortly ellipsoidal. Does not produce fermentation in grape must. Its microscopical appearance is given in figure 1.

*Dematium Variety*.—Grows vigorously in must giving a slimy mass of colorless cells and a surface growth of black cells arranged in long chains. Found in large numbers on grapes examined in 1912. Does not cause fermentation in must. Microscopical appearance is given in figure 2.

*Monilia Species*.—Colonies on agar resemble yeast colonies with rootlike projections into the depths of the medium. Gives a feeble fermentation in grape must, followed by a mycelial growth on the surface of the liquid; in old cultures, part of the mycelium becomes olive green (fig. 2).

*Dark Green Mold* (unidentified).—Found growing profusely as a parasitic fungus on skins of grapes after the early rains in fall of 1912; grows equally well on artificial media in the laboratory, so may be termed a facultative parasite. It causes no visible fermentation in must and is odorless. Its microscopical appearance is given in figure 1. It may possibly be a *Cladosporium* form.

## BACTERIA

*Vinegar Bacteria*.—The only forms of bacteria so far found on grapes examined at the laboratory or winery have been vinegar bacteria. They were observed on two different lots of grapes, both of which had been shipped long distances and were in a badly broken and moldy condition. Both cultures grew rapidly in grape must giving a heavy tough film. One culture gave bacteria of medium length that tended to form in chains, while the other developed in the form of very short rods usually grouped in pairs (fig. 2).

“*Tourne*” *Bacteria*.—The form shown in figure 2 is found in wines quite often, but has not been met with on California grapes. It grows in the absence of air and gives a “mousey” taste to badly affected wines (fig. 2). This organism is otherwise known as *Bacterium mannito-pocum*. It occurs in vinegar and fermented fruit juices.

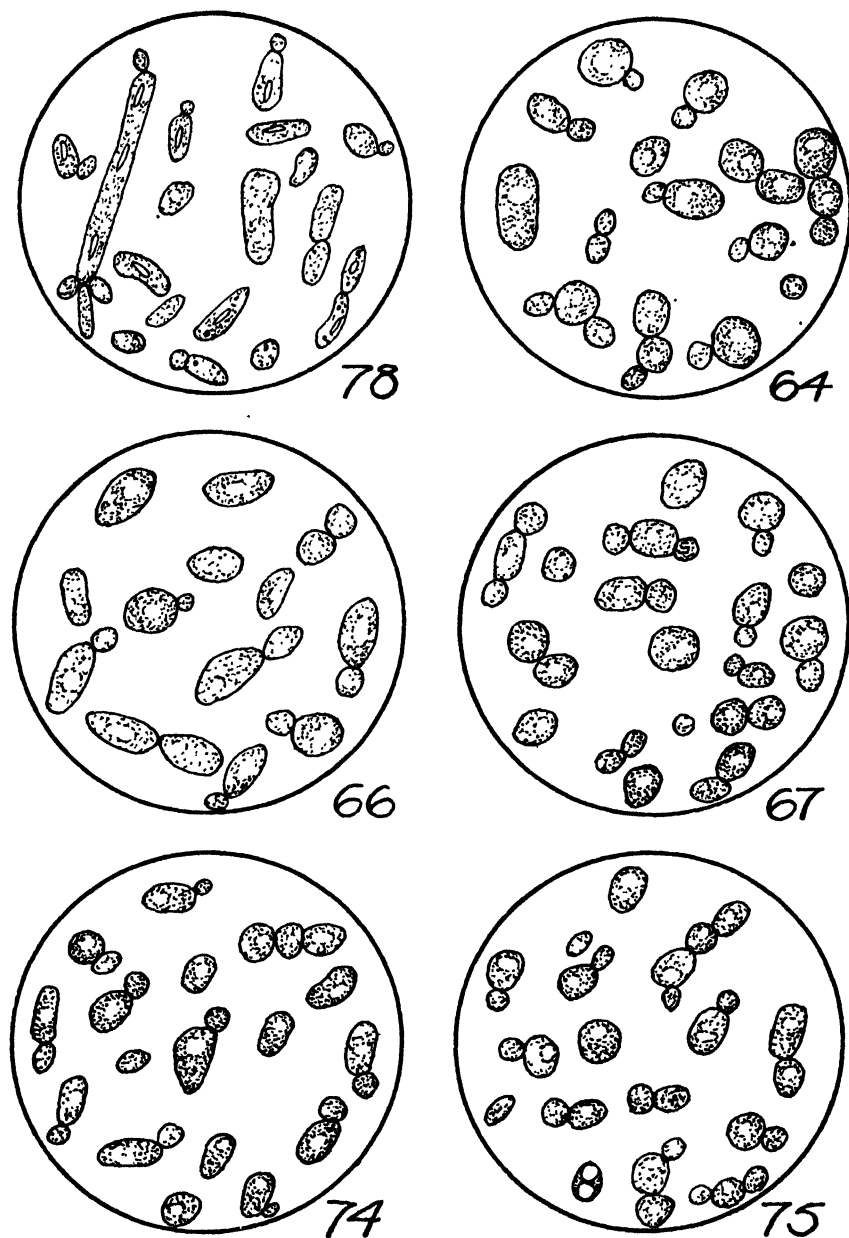


Figure 5

Yeasts from California grapes:

- 78. *Mycoderma* yeast from Contra Costa County grapes.
- 64. *S. ellipsoideus* from Acampo grapes.
- 66. *S. ellipsoideus* from Davis grapes.
- 67. *S. ellipsoideus* from Contra Costa County grapes.
- 74. *S. ellipsoideus* from Acampo grapes.
- 75. *S. ellipsoideus* from Contra Costa County grapes.

### III. CHARACTERISTICS OF YEASTS FROM CALIFORNIA GRAPES

#### METHODS OF STUDY

The yeasts isolated from grapes in 1911 according to the methods given on preceding pages were studied in accordance with the following outline. From the tests made, it was possible to determine the specific character of the yeasts and to obtain a fair idea as to their suitability or unsuitability for wine making purposes.

*Morphology.*—(1) Macroscopical Appearance.—In must, beer wort, nutrient dextrose, saccharose, and lactose solutions; on agar must and on gelatin must.

(2) Microscopical Appearance.—Size, form, and general appearance from five days' growth in grape must. Microscopical appearance of agar colonies.

(3) Spore Formation.—Observed by placing the sediment from vigorous cultures on gypsum blocks partly immersed in water in wide mouthed bottles plugged with cotton wool. This arrangement gives the necessary conditions of aeration, poor food supply, and moisture. Tests were made of spore formation at 22° C and 28° C

*Fermentation Tests.*—(1) Rates of Fermentation.—The rates of fermentation in grape musts of 23.01% and 29.75% Balling, in beer wort, nutrient<sup>3</sup> dextrose solution, nutrient saccharose solution and nutrient lactose solution were determined on 100 c.c. portions of the above liquids by noting the loss in weight during fermentation after inoculation with pure cultures of the organism under observation. The tests were carried out at 33° C. The loss in weight is due to the formation of carbon dioxide during fermentation. The escape of this gas causes the change in weight by which the fermentation may be followed. The main chemical reaction involved is the following:



A check flask, which was not inoculated, was used as a means of obtaining the loss in weight due to evaporation. The figures given in the tables are corrected for this loss. Points representing the time in hours reckoned from the time of inoculation and also representing the

<sup>3</sup> The nutrient solutions used for the dextrose, saccharose, and lactose consisted of .01 grams magnesium sulfate, .5 grams of dipotassium phosphate, 10 grams Witte's pepton dissolved in 1000 c.c. of water. To a portion of this solution was added 15% dextrose, to a second part 15% saccharose, and to the third, 15% lactose.

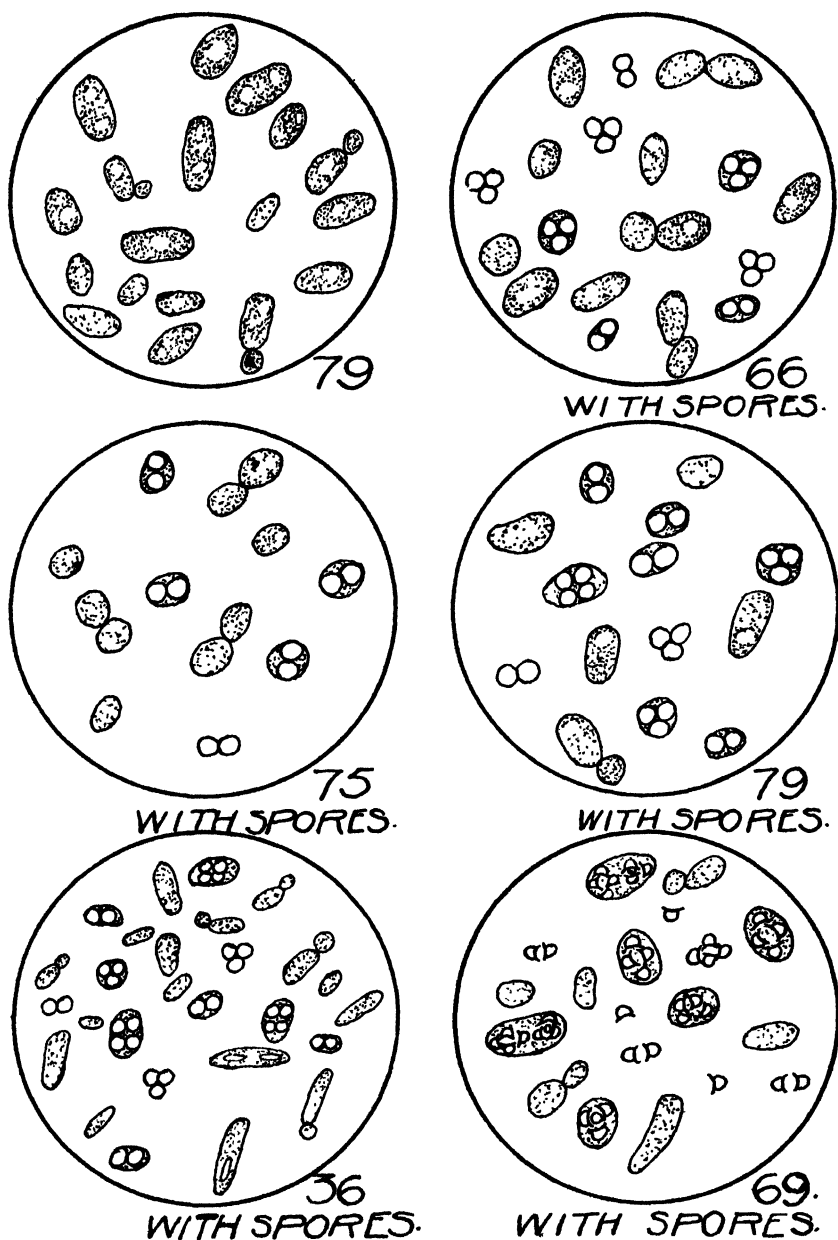


Figure 6

Yeasts from California grapes showing spore formation:

79. *S. ellipsoideus* from Contra Costa County, California.

66. *S. ellipsoideus* from Davis grapes showing spores.

75. *S. ellipsoideus* from Contra Costa County grapes, showing spores.

79. *S. ellipsoideus* from Contra Costa County grapes, showing spores.

36. *S. pastorianus* from Davis grapes, showing spores.

69. *Willia* from Acampo grapes, showing spores.

loss in weight have been plotted on diagrams and the individual points connected to give "fermentation curves." These curves show at a glance the character of the fermentations and serve as convenient means of comparison.

(1) Products of Fermentation.—The products of fermentation in the solutions given above were determined after the rates of fermentation were ascertained. Similar tests were made in grape must of 30°<sup>4</sup> Balling fermented by the various yeasts at 24° C.

#### YEASTS STUDIED

The yeasts have been taken up in the following order: (1) *S. apiculatus* yeasts, (2) *Mycoderma* forms, (3) *Torula* yeasts, (4) *S. pastorianus* and *Willia* yeasts, and (5) *S. ellipsoideus* yeasts. The characteristics of each have been discussed according to the outline given above.

#### APICULATUS YEASTS FROM CALIFORNIA GRAPES

Two *apiculatus* yeasts were studied. One of these was isolated from grapes grown at Davis and the other from grapes grown at Acampo. The morphology and biological characteristics were studied as previously outlined.

Microscopical appearance is based on cells from five days' growth in grape must of 15% Balling. The agar used for plate cultures consisted of 2 grams of agar agar, 60 c.c. of must, and 1000 c.c. of water. The gelatin medium consisted of 15 grams of gelatin to each 100 c.c. of grape must.

#### *Organism 35. Apiculatus from Grapes Grown at Davis*

*Morphology.*—Microscopical Appearance.—Majority of cells lemon-shape. Cells broader and larger per cent apiculate-shape than in case of yeast no. 72.

*Size.*—Average,  $4.5\mu \times 2.4\mu$ . Maximum,  $7.5\mu \times 4.5\mu$ . Minimum,  $3\mu \times 1.5\mu$ .

*Spore Formation.*—No spores at 22° C or 28° C.

*Colonies on Agar Agar.*—Flat. Elevation uniform. Smaller than *S. ellipsoideus* colonies. Semitranslucent. Edges smooth and entire. Under microscope the edges of the colonies are entire.

<sup>4</sup>Degrees Balling indicates total dissolved solids in grams per 100 grams, as cane sugar.

Colonies on Gelatin.—Small. Gelatin is slowly liquefied, giving craterlike depressions. Edges entire.

Growth in Liquid Media.—In grape must, beer wort, nutrient dextrose, and saccharose solutions, rapid but fermentation feeble. Growth in lactose slight and no fermentation. Sediment and growth in liquid, fine grained. Cells settle slowly. Liquid becomes fruity in flavor and odor.

*Organism 72. Apiculatus from Grapes Grown at Acampo*

*Morphology.*—Microscopical Appearance.—Majority of cells sausage-shaped or spherical. Only a few typical apiculate cells. Decidedly different from no. 35.

Size.—Average,  $4.5\mu \times 2.7\mu$ . Maximum,  $6\mu \times 2.7\mu$ . Minimum  $2.2\mu \times 1.5\mu$ .

Spore Formation.—No spores at  $28^{\circ}\text{C}$  or  $22^{\circ}\text{C}$ .

Colonies on Agar Agar.—Small. Edges undulate or indented. Centers slightly elevated. Opaque and porcelainous. Under low power, edges entire. Different from no. 35.

Colonies on Gelatin.—Small. Edges branchlike. Waxy luster. Edges under microscope lacerate, *i.e.*, saw-tooth appearance. Gelatin slowly liquefied to form craterlike depressions around colonies; whole plate liquefied in few days.

Growth in Liquid Media.—In grape must, beer wort, and nutrient dextrose, growth is rapid. Almost no growth in saccharose and lactose nutrient solutions. Deposit and growth in liquid fine grained. Settles very slowly. Fruity odor and flavor in fermented liquid.

*Rates of Fermentation, Various Media.*

The following solutions were used:

- (a) Grape must,  $23.01^{\circ}$  Balling.
- (b) Grape must,  $29.75^{\circ}$  Balling.
- (c) Sweet beer wort,  $14^{\circ}$  Balling.
- (d)<sup>5</sup> Nutrient dextrose, 15% dextrose.
- (e) Nutrient saccharose, 15% saccharose.
- (f) Nutrient lactose, 15% saccharose.

<sup>5</sup> The nutrient solutions (d), (e), (f), contained .01 grams  $\text{MgSO}_4$ , .5 grams  $\text{K}_2\text{HPO}_4$ , 10 grams Witte's pepton, per 1000 c.c. water, and 150 grams of the respective sugars per 1000 c.c.

One hundred cubic centimeters of the above solutions were sterilized in small plugged flasks. The solutions were inoculated in each instance with one platinum loopful of a vigorous culture. Non-inoculated checks were used to correct for loss due to evaporation. The inoculated flasks were placed in an incubator at 33° C.

The flasks were weighed at intervals during fermentation. The loss in weight corrected for loss due to evaporation was taken as a measure of the rate of fermentation.

TABLE 1  
DATA ON RATES OF FERMENTATION OF YEASTS NUMBERS 35 AND 72 (*S. apiculatus*)  
IN VARIOUS SUGAR SOLUTIONS

Yeast number	Time in hours	Must of 23° Balling, loss in grams	Concentrated must, loss in grams	Wort loss, in grams	Dextrose loss, in grams	Saccharose loss, in grams	Lactose loss, in grams
35	46	1.85	.33	.35	.45	.03	.00
35	73	2.84	1.10	.45	.65	.02	.00
35	96	3.26	2.05	.47	.79	.01	.00
35	118	3.42	2.53	.47	.93	.01	.00
35	167	3.54	2.90	.49	1.13	.00	.00
35	215	3.54	2.95	.57	1.35	.03	.00
35	239	3.57	2.98	.58	1.40	.00	.00
72	27	.18	.01	.35	.10	.00	.00
72	52	1.09	.19	.56	.33	.00	.00
72	77	1.92	.42	.59	.83	.00	.00
72	125	2.75	1.15	.69	.98	.00	.00
72	167	2.84	1.32	.96	.....	.00	.00
72	244	3.18	2.05	.97	1.21	.00	.00
72	341	3.50	2.47	1.10	1.42	.00	.00
72	421	3.55	2.53	1.13	1.44	.00	.00

From an examination of the data given in table 1 and of the fermentation curves in the accompanying diagram, it is evident that both yeasts have very low fermenting power and slow rates of fermentation. In grape must at 33° C, yeast no. 35 produces a more rapid fermentation than yeast no. 72, but in beer wort and dextrose solutions the two yeasts are similar. The concentrated must of 29.75° Balling has a greater retarding effect on yeast no. 72 than on yeast no. 35. Neither yeast fermented saccharose or lactose. Because of their difference of morphology and in the character of their fermentations, it is justifiable to designate them as two different varieties of *apiculatus* yeast.

*Attenuation and Alcohol Formation.*—The liquids listed in table 2 were analyzed after fermentation. The term "attenuation" in the accompanying table refers to the loss in sugar during fermentation. The alcohol, theoretical and observed, is reported as volume per cent.

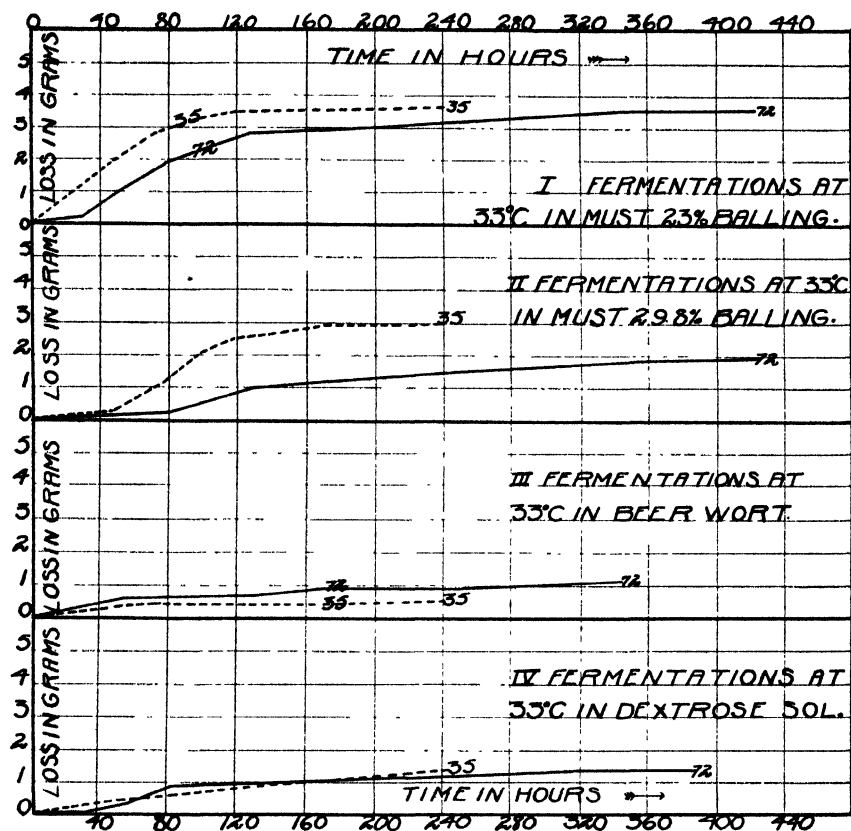
Fig. 7.—Fermentation curves of *S. apiculatus* yeasts from California grapes.

TABLE 2

YEASTS NUMBERS 35 AND 72 (*S. apiculatus*). ATTENUATION AND YIELDS OF ALCOHOL IN MUST, BEER WORT, DEXTROSE, SACCHAROSE, AND LACTOSE SOLUTIONS

Yeast No.	Medium	Attenuation in grams per 100 c. c.	Theoretical yield of alcohol, per cent	Observed yield of alcohol, per cent	Per cent of theoretical yield obtained
72	Must of 23° Balling .....	3.81	2.50	1.00	40.0
72	Must of 29°75 Balling .....	1.20	.79	.30	37.9
72	Beer wort of 14°56 Balling .....	1.61	1.11	.30	27.6
72	Dextrose solution, 14°9 Ball. ....	2.73	1.79	.65	36.3
72	Saccharose sol., 13°73 Ball. ....	.78	.53	.00	.00
72	Lactose solution, 15°16 Ball. ....	.31	.20	.00	.00
35	Must of 23° Balling .....	8.50	5.58	3.05	54.5
35	Must of 29°75 Balling .....	6.15	4.03	2.35	58.3
35	Beer wort of 14°56 Balling .....	1.35	.93	.....	.....
35	Dextrose solution, 14°9 Ball. ....	3.25	2.13	.65	30.5
35	Saccharose sol., 13°73 Ball. ....	.00	.00	.00	.....
35	Lactose solution, 15°16 Ball. ....	.00	.00	.00	.....



The results show that in most cases, less than 50% of the alcohol, theoretically obtainable from the sugar fermented, was actually formed by the two yeasts. For example, yeast no. 35 destroyed 3.81% sugar and should have produced 2.5% alcohol in must of 23° Balling, while the actual yield was only 1% or 40% of the amount that was theoretically possible. True wine yeasts of the *S. ellipsoideus* type produce at least 90% of the theoretical yield in grape must.

Since the yields of alcohol are so low and the flavor of the fermented liquids is not desirable, the two yeasts may be considered of little use for industrial purposes. Their importance lies in their power to cause trouble in wine making by growing in natural fermentations before the true wine yeast has developed. In this way they may injure the flavor and clearing quality of the wine and favor "stuck" tanks by the production of compounds that are injurious to the *S. ellipsoideus* yeast. Because of their very common occurrence in large numbers on California grapes, measures should always be taken to discourage their growth.

#### MYCODERMA FORMS FROM CALIFORNIA GRAPES

Under *Mycoderma* forms, seven film forming organisms have been described in the following pages. Some of these organisms are yeast-like in appearance and in their fermentation properties; other resemble molds more closely than they do yeasts. All have the common properties of forming heavy films on nutrient liquids and of not forming spores.

##### *Organism 65. Mycoderma from Acampo Grapes.*

*Morphology.*—Microscopical Appearance.—Sausage-shape to filamentous. Tends to grow in mycelial threads (fig. 4).

Size.—Average,  $9\mu \times 2.5\mu$ . Maximum,  $40\mu \times 2.5\mu$ . Minimum,  $2\mu \times 1.5\mu$ .

Spore Formation.—No spores at 22° C and 28° C.

Colonies on Agar Agar.—Small and growth slow. Powdery appearance. Color, white. Flat. Growth at surface only. Edges made up of mycelial threads. Under low power, colonies are made up of chains of long cells. These spread over the surface of the agar.

Colonies on Gelatin.—More vigorous than on agar. Concentric ring growth. Powdery white. Flat. Under microscope, same as on agar. Gelatin slowly liquefied.

Growth on Liquid Media.—Grows profusely on surface of grape must, beer wort, dextrose, saccharose and lactose solutions, but does not produce visible fermentation in any of these liquids. Film remains permanently on the surface. Does not sink. Film is very tough in texture.

*Organism 68. Mycoderma from Grapes Grown at Acampo, California*

*Morphology.*—Microscopical Appearance.—Predominating form sausage-shaped. Cells vary from spherical to long-rod shaped cells (fig. 4).

*Size.*—Average,  $7.5\mu \times 3.75\mu$ . Maximum,  $15\mu \times 3.75\mu$ . Minimum,  $3\mu \times 3\mu$ .

*Spore Formation.*—No spores formed at  $22^{\circ}\text{C}$  or  $28^{\circ}\text{C}$ .

*Colonies on Agar Agar.*—Large. Edges entire. Waxy luster. Centers of colonies slightly elevated. Edges entire. Opaque. Under low power of microscope, internal structure granular and edges are surrounded by long chains of cells of ellipsoidal shape.

*Colonies on Gelatin.* Filmy, translucent colonies, that rapidly cover the whole surface. Under microscope the colonies are made up of chains of cells.

*Growth in Liquid Media.*—Heavy growth in grape must, beer wort, and dextrose with formation of large sediment, the volume of which may be equal to 10 per cent of the total volume of the liquid. Growth slow in lactose and saccharose solutions. Visible fermentation very slow in grape must, beer wort and dextrose solution at first, but finally becomes vigorous in must and dextrose solutions. No visible fermentation in lactose and saccharose.

*Organism 70. Mycoderma from Acampo Grapes*

*Morphology.*—Microscopical Appearance.—Usual form, sausage-shape, but vary from spherical to sausage-shaped. Cells are usually grouped in branching chains (fig. 4).

*Size.*—Average,  $7\mu \times 2\mu$ . Maximum,  $18\mu \times 2\mu$ . Minimum,  $1.5\mu \times 1.5\mu$ .

*Spore Formation.*—No spores formed at  $22^{\circ}\text{C}$  and  $28^{\circ}\text{C}$ .

*Colonies on Agar Agar.* Large. Center elevated. Surface corrugated by ridges radiating from centers of colonies. Centers of colonies brown; edges yellowish white. Edges indented. Low power of microscope shows chains of cells growing from edges of colonies; this growth is beneath the surface of the agar (see plate 2).

Colonies on Gelatin.—Large. Centers convex. Colonies flatten near edges and become filmy in appearance. Edges entire under microscope. Gelatin slowly liquefied.

Growth in Liquid Media.—Heavy, wrinkled surface growth which frequently falls to bottom forming voluminous sediment. Fermentation slow. No fermentation in lactose.

*Organism 71. Mycoderma from Contra Costa County Grapes*

*Morphology.*—Microscopical Appearance.—Predominating form sausage-shaped. Varies from spherical to sausage-shape (fig. 4).

Size.—Average,  $6.9\mu \times 2\mu$ . Maximum,  $18\mu \times 2\mu$ . Minimum,  $1.5\mu \times 1.5\mu$ .

Spore Formation.—No spores at  $22^{\circ}\text{C}$  or  $28^{\circ}\text{C}$ .

Colonies on Agar Agar.—Large. Flat. Spreading. Edges lobate. Edges entire under microscope (see plate 2).

Colonies on Gelatin.—Flat. Filmy. Translucent. Rapidly cover entire surface of the gelatin. Slow liquefaction (see plate 2).

Growth in Liquid Media.—A wrinkled, loosely coherent film on all liquids tested. Films frequently sink and are quickly replaced by new growth. Fermentation in must, beer wort, and dextrose fairly vigorous, but extends over long period. No perceptible fermentation in saccharose and lactose.

*Organism 73. Mycoderma from Grapes Grown at Acampo*

*Morphology.*—Microscopical Appearance.—Usual form sausage-shaped. Varies from spherical to sausage-shaped (fig. 4).

Size.—Average,  $6.7\mu \times 2.5\mu$ . Maximum,  $18\mu \times 3\mu$ . Minimum,  $3\mu \times 1.5\mu$ .

Spore Formation.—No spore formation at  $22^{\circ}\text{C}$  and  $28^{\circ}\text{C}$ .

Colonies on Agar Agar.—Centers elevated. Edges flat and serrated. Shape irregular. Under the microscope chains of cells are seen to radiate from the edges of the colonies. The chains are made up of long cells with pairs of short ellipsoidal cells at the junctures of the long cells.

Colonies on Gelatin.—Flat. Smooth. Filmy. Translucent. Gelatin rapidly covered by a filamentous growth. Gelatin is slowly softened to a syrupy consistency.

Growth in Liquid Media.—Growth vigorous in all liquid media tested. Vigorous fermentation in grape must and dextrose solution.

During fermentation a heavy wrinkled film is formed with a moderate amount of sediment growth. After fermentation most of the film growth settles to the bottom. Fermentation is more vigorous and film formation is not so well developed as in no. 70.

*Organism 76. Mycoderma from Grapes Grown at Acampo*

*Morphology.*—Microscopical Appearance.—Sausage-shaped to filamentous (fig. 4).

Size.—Average,  $10.5\mu \times 2.1\mu$ . Maximum,  $75\mu \times 2.5\mu$ . Minimum,  $3\mu \times 2\mu$ .

Spore Formation.—No spores formed at  $22^{\circ}\text{C}$  or  $28^{\circ}\text{C}$ .

Colonies on Agar Agar.—Large. Flat. Surface smooth. Centers slightly elevated. Edges smooth and entire under microscope.

Colonies on Gelatin.—Large. Resinous. Translucent. Surface of colonies deeply corrugated. Mycelial projections from edges visible to naked eye. Surface glistening and slimy. Under microscope edges are fringed by branching chains of very long cells.

Growth in Liquid Media.—Growth in all media tested consists first of powdery white colonies. These coalesce to form a resinous heavy film resembling a growth of vinegar bacteria. The growth sinks after several weeks and is replaced by a second film. No visible fermentation in any liquids tested.

*Organism 78. Mycoderma from Contra Costa County Grapes*

*Morphology.*—Microscopical Appearance.—Sausage-shaped cells predominate. Very long filamentous cells not uncommon (fig. 4).

Size.—Average,  $10.5\mu \times 3\mu$ . Maximum,  $37.5\mu \times 3\mu$ . Minimum,  $4.5\mu \times 2\mu$ .

Spore Formation.—No spores at  $22^{\circ}\text{C}$  and  $28^{\circ}\text{C}$ .

Colonies on Agar Agar.—Large and flat. Centers slightly elevated. Waxy luster. Edges show hazy growth of mycelium. Under microscope colonies are seen to be surrounded by radiating mycelial threads.

Colonies on Gelatin.—Large. Flat. Smooth. Translucent. Rapidly cover the entire surface of the gelatin. Gelatin rapidly softened to a syrupy consistency (see plate 2).

Growth in Liquid Media.—In grape must, beer wort, and dextrose solutions, growth is rapid and fermentation vigorous; in saccharose growth vigorous and fermentation slow; little growth in lactose. Consists chiefly of surface growth. Small sediment.

*Fermentation Tests of Mycoderma Forms from California Grapes.*

The data obtained by noting the loss in weight at 33° C of flasks containing 100 c.c. of grape must 23.01° Balling, grape must 29.8° Balling, beer wort 14° Balling, dextrose 15° Balling, saccharose 15° Balling, and lactose 15° Balling solutions, respectively, after inoculation with pure cultures of the above yeasts appear in table 3. The attenuation, representing the sugar destroyed, together with the yields of alcohol in the various sugar solutions tested are given in table 3. After each table the results of the tests are discussed. The nutrient solutions used were the same as those described for yeasts 35 and 72. See description of apiculatus yeasts.

TABLE 3

## FERMENTATION TESTS OF MYCODERMA FORMS FROM CALIFORNIA GRAPES

Yeast number	Time in hours	Must of 23° Balling, loss in grams	Must of 29° Balling, loss, in grams	Wort loss, in grams 14 per cent solution	Dextrose loss, in grams 15 per cent solution	Saccharose loss, in grams 15 per cent solution	Lactose loss, in grams 15 per cent solution
68	18	.00	.00	.04	.07	.00	.00
68	42	.70	.01	.21	.31	.10	.00
68	66	1.51	.24	.38	.93	.10	.00
68	92	2.45	.62	.37	2.10	.10	.00
68	123	3.35	1.32	.38	2.90	.10	.00
68	165	5.80	2.00	.40	5.89	.10	.03
68	236	8.45	3.30	.42	6.40	.10	.03
68	284	8.48	3.80	.50	6.40	.10	.03
68	314	8.48	3.80	.51	.....	.10	.03
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65	18	.00	.00	.00	.00	.00	.00
65	42	.03	.00	.00	.00	.01	.00
65	66	.03	.00	.03	.00	.01	.00
65	92	.03	.00	.08	.04	.01	.00
65	123	.03	.04	.08	.09	.07	.03
65	165	.03	.04	.12	.09	.07	.03
65	236	.03	.04	.17	.09	.07	.03
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70	18	.02	.03	.00	.10	.00	.00
70	42	.10	.05	.06	.18	.20	.00
70	66	.38	.08	.33	.41	.20	.06
70	92	.80	.....	.38	.42	.25	.05
70	123	1.90	.08	.80	1.30	.60	.20
70	165	2.47	.83	1.00	1.65	.90	.20
70	236	2.57	.95	1.00	1.65	.90	.30
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71	46	.85	.35	.25	.....	.00	.00
71	73	1.85	1.17	.34	.46	.00	.....
71	96	2.70	2.19	.41	.....	.00	.....
71	118	3.74	3.19	.42	2.31	.01	.....
71	167	5.47	3.89	.42	4.20	.00	.00
71	215	6.40	5.90	.44	.....	.04	.....
71	239	6.85	6.40	.45	.....	.04	.....

TABLE 3—(Continued)

Yeast number	Time in hours	Must of 28° Balling, loss in grams	Must of 29° Balling, loss in grams	Wort loss, in grams 14 per cent solution	Dextrose loss, in grams 15 per cent solution	Saccharose loss, in grams 15 per cent solution	Lactose loss, in grams 15 per cent solution
73	27	.20	.06	.18	.66	.10	.00
73	52	1.09	.61	.34	1.79	.28	.00
73	77	2.26	1.56	.49	3.21	.58	.00
73	125	4.14	3.34	.59	4.11	.93	.00
73	167	5.69	4.59	.61	5.72	1.19	.00
73	244	9.25	6.62	.72	7.07	1.41	.00
73	341	9.32	8.24	.87	7.35	1.52	.00
73	421	9.52	9.65	.92	.....	.....	.00
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76	27	.00	.04	.00	.00	.00	.00
76	52	.04	.08	.04	.07	.00	.....
76	77	.....	.....	.....	.....	.00	.....
76	125	.....	.....	.....	.....	.00	.....
76	167	.31	.13	.24	.20	.00	.....
76	244	.42	.39	.46	.41	.00	.....
76	341	.62	.69	.62	.50	.00	.....
76	421	.64	.69	.70	.54	.00	.....
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78	27	.23	.16	.23	.38	.23	.00
78	52	1.12	.44	.54	.80	.54	.00
78	77	2.37	1.64	1.75	2.05	1.37	.00
78	125	4.34	3.29	3.24	3.38	1.74	.00
78	167	6.05	4.60	4.52	.....	1.95	.00
78	244	7.55	6.20	5.25	6.16	2.26	.00
78	341	9.50	7.50	.....	6.98	2.50	.00
78	421	9.85	8.05	.....	7.29	2.70	.00

The seven different varieties of mycoderma yeasts, whose rates of fermentation in normal must, concentrated must, beer wort, dextrose, saccharose and lactose solutions were studied gave great variation in the rate of fermentation and the amounts of sugar fermented. Organism 65 did not cause fermentation in any of the liquids tested. though it gave a vigorous growth. On the other hand, yeasts 68, 73, and 78, developed fairly strong fermentations in must and beer wort, destroying nearly as much sugar as the true wine yeasts. Organism 76, caused a very feeble fermentation in grape must but did not ferment any of the other liquids tested. The mycoderma forms did not ferment so rapidly in dextrose solution as in grape must. Contrasted with the mycoderma forms the true wine yeasts did not exhibit this difference. Lactose was not fermented by any of the mycoderma forms. Saccharose was attacked to a slight degree by forms 70, 73, and 78, the amount of sugar actually fermented in each case being small. In general, the fermentation curves of the forms shown in the diagrams

differ from those of the true wine yeasts shown in figures 12 to 15, by their low and gentle slope throughout. In most cases they show a slower start of fermentation than do the *S. ellipsoideus* yeasts. Be-

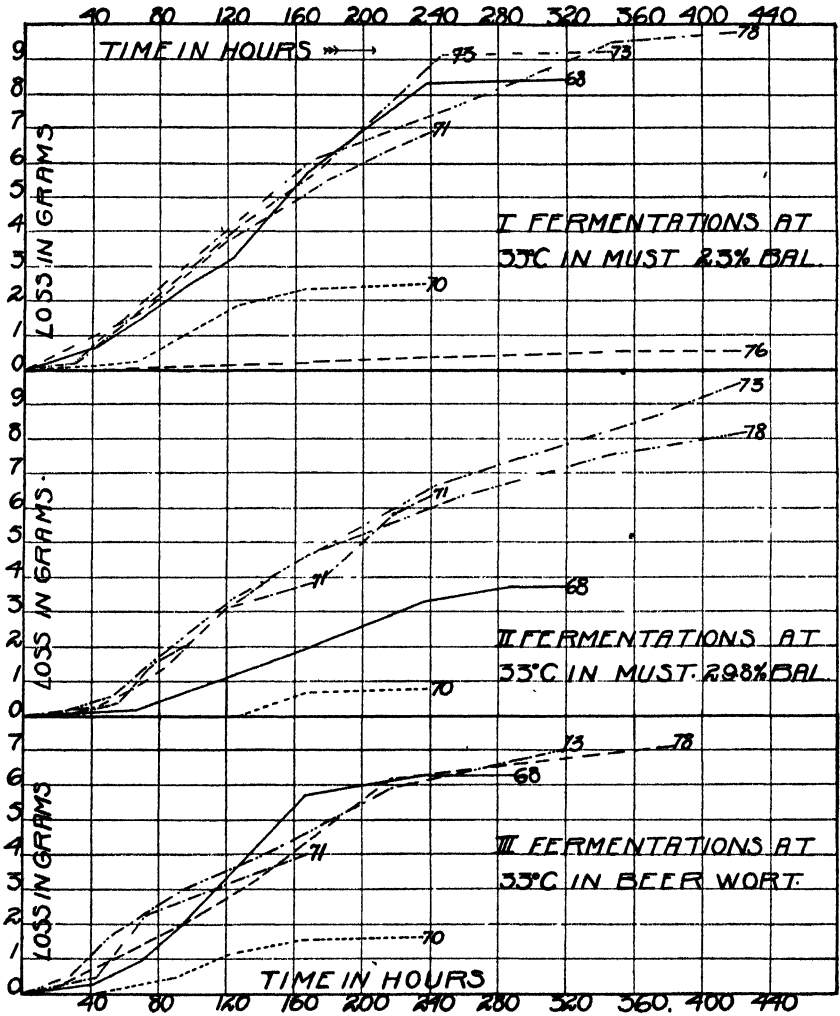


Fig. 8.—Fermentation curves of *Mycoderma* forms in must of 23° and 29°8 Balling and beer wort.

cause of their slow and incomplete fermentations the mycoderma forms discussed above are not desirable types to have in wine fermentations.

*Attenuation and Alcohol Formation.*—Analyses were made of the liquids fermented by the mycoderma forms (table 4).

TABLE 4

YEASTS NUMBERS 65, 68, 70, 71, 73, 76, AND 78. ATTENUATION AND YIELDS OF ALCOHOL IN MUST, BEER WORT, DEXTROSE, SACCHAROSE, AND

## LACTOSE SOLUTIONS

Yeast No.	Medium	Attenuation in grams per 100 c c	Theoretical yield of alcohol	Observed yield of alcohol	Per cent of theoretical yield obtained
64	Must of 23° Balling .....	.....	.....	0.00	.....
65	Must of 29°8 Balling .....	.....	.....	0.00	.....
65	Beer wort of 14° Balling .....	.....	.....	0.00	.....
65	Dextrose solution, 15% .....	.....	.....	0.00	.....
65	Saccharose solution, 15% ....	.....	.....	0.00	.....
65	Lactose solution, 15% .....	.....	.....	0.00	.....
68	Must of 23° Balling .....	19.88	13.08	7.45	56.9
68	Must of 32° Balling .....	7.84	5.15	4.50	89.3
68	Beer wort of 14° Balling ....	.86	.62	.60	96.8
68	Dextrose solution, 15% .....	12.68	8.30	6.40	77.1
68	Saccharose solution, 15% ....	.03	.02	0.00	00.0
68	Lactose solution, 15% .....	.27	.19	0.00	00.0
70	Must of 23° Balling .....	6.66	4.38	2.55	58.2
70	Must of 29°8 Balling .....	2.05	1.35	1.00	74.5
70	Beer wort of 14° Balling ....	1.43	.96	.80	83.3
70	Dextrose solution, 15% .....	4.51	2.96	1.70	57.4
70	Saccharose solution, 15% ....	.....	.....	.....	.....
70	Lactose solution, 15% .....	0.00	0.00	0.00	00.0
71	Must of 23° Balling .....	17.61	11.58	8.80	75.9
71	Must of 29°8 Balling .....	.....	.....	7.90	.....
71	Beer wort of 14° Balling ....	2.91	2.03	0.00	00.0
71	Dextrose solution, 15% .....	12.20	8.02	4.10	51.1
71	Saccharose solution, 15% ....	.08	.05	0.00	00.0
71	Lactose solution, 15% .....	.16	.11	0.00	00.0
73	Must of 23° Balling .....	12.01	7.90	7.50	94.9
73	Must of 29°8 Balling .....	14.75	9.70	6.80	70.1
73	Beer wort of 14° Balling ....	1.61	1.16	.52	44.8
73	Dextrose solution, 15% .....	12.60	8.29	5.60	67.5
73	Saccharose solution, 15% ....	2.08	1.50	1.50	100
73	Lactose solution, 15% .....	.35	.25	0.00	00.0
76	Must of 23° Balling .....	.....	.....	0.00	.....
76	Must of 29°8 Balling .....	.....	.....	0.00	.....
76	Beer wort of 14° Balling ....	.....	.....	0.00	.....
76	Dextrose solution, 15% .....	.....	.....	0.00	.....
76	Saccharose solution, 15% ....	.....	.....	0.00	.....
76	Lactose solution, 15% .....	.....	.....	0.00	.....
78	Must of 23° Balling .....	18.87	12.41	10.30	83.1
78	Must of 29°8 Balling .....	14.13	9.29	9.00	97.9
78	Beer wort of 14° Balling ....	12.15	7.90	7.20	91.1
78	Dextrose solution, 15% .....	13.22	8.69	6.20	71.3
78	Saccharose solution, 15% ....	3.93	2.86	1.40	48.9
78	Lactose solution. 15% .....	.36	.26	0.00	00.0



An examination of the data given in table 4 shows that in grape must, which was the most favorable medium for the activity of the yeasts under observation, amounts of alcohol varying from .00% to 10.3% were formed. Some of the organisms, namely, 73 and 78, gave good yields of alcohol for the sugar fermented in grape must, while others e.g., 68, 70, 71, gave low yields. Yeasts 65 and 76 gave no alcohol in any of the liquids in which they were grown, although they destroyed small amounts of sugar in some of the solutions. None of the organisms of this group fermented beer wort to any notable degree and the fermentations that did take place in this liquid probably represented the destruction of dextrose present, only, while it is probable that the maltose was not attacked. Saccharose likewise proved very resistant to fermentation by the above forms. Organisms 68, 71, 73, and 78 formed considerable amounts of alcohol in dextrose solution, indicating that this sugar is more easily attacked than cane sugar or maltose. These facts indicate that the yeasts under discussion are deficient in invertase and maltase, enzymes that are necessary to the fermentation of saccharose and maltose.

Since the mycoderma forms discussed above do not ferment beer wort or cane sugar, they would be useless in the manufacture of beer or of alcohol from materials containing cane sugar. The amounts of alcohol formed in grape must by several of the organisms might be sufficient for some purposes, but their use for the production of wine is excluded by the fact that all of them produce disagreeable tastes and odors in grape must, resulting in the formation of an undrinkable fermented liquid in all cases. Therefore, it is to the winemaker's interest to prevent the growth of these organisms.

#### TORULA FORMS FROM CALIFORNIA GRAPES

Two yeasts of the torula type were found on grapes examined in 1911, one from Davis grapes and the other from grapes grown in Contra Costa County, California.

##### *Organism 37. Torula Yeast from Grapes Grown at Davis*

*Morphology.*—Microscopical Appearance.—Varies from spherical to shortly ellipsoidal in form; latter form predominating. Cells tend to form chains of cells of various sizes (see fig. 3).

*Size.*—Average,  $4.6\mu \times 3.7\mu$ . Maximum,  $15\mu \times 6\mu$ . Minimum,  $3\mu \times 3\mu$ .

*Spore Formation.*—No spores formed at 22° C or 28° C.

Colonies on Agar Agar.—Almost flat, but centers, slightly elevated. White. Waxy luster. Edges entire. Colonies in the agar, spherical. Under microscope, edges of colonies entire.

Colonies on Gelatin.—Small. White. Waxy luster. Gelatin quickly liquefied.

Growth in Liquid Media.—Growth in grape must, dextrose, and beer wort, rapid, with formation of considerable sediment, and development of a feeble bottom fermentation. No film formation. No fermentation in lactose solutions, but good growth. Feeble fermentation and good growth in saccharose. Rancid taste and odor developed in fermented liquids.

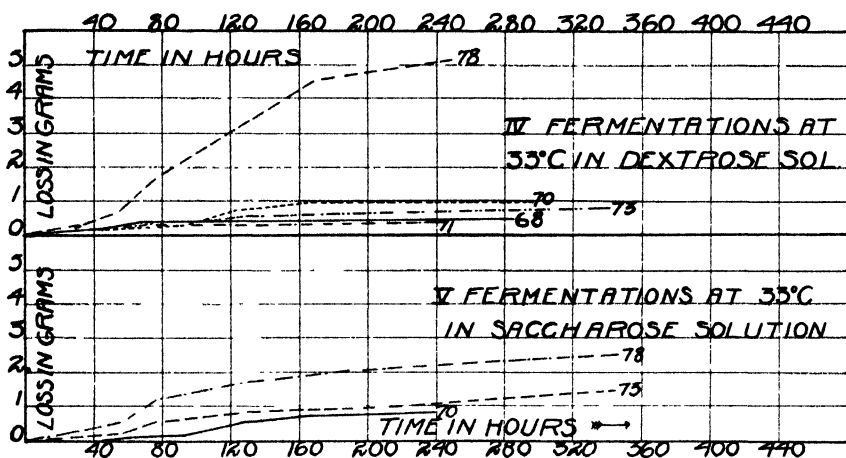


Fig. 9.—Fermentation curves of *Mycoderma* forms in dextrose and saccharose solutions.

#### *Organism 77. Torula from Contra Costa County Grapes*

*Morphology.*—Microscopical Appearance.—Vary from ellipsoidal to sausage-shaped with ellipsoidal form predominating (see fig. 3). Tends to form short chains.

*Size.*—Average,  $4\mu \times 2.7\mu$ . Maximum,  $8\mu \times 4.5\mu$ . Minimum,  $2.2\mu \times 1.5\mu$ .

Colonies on Agar Agar.—Small. White. Convex. Waxy luster. Spherical colonies in the agar. Under the microscope colonies are found to be surrounded by straight chains of long cells. Most other yeasts examined that formed side chains in this manner produced branching cells.

Colonies on Gelatin.—Contoured surface with highest elevation at centers of colonies. Centers of colonies colorless. Chalklike at edges.

Undermicroscope, colonies are fringed with branching chains of long cells.

Growth in Liquid Media.—Growth in all liquids slow. Feeble fermentation in grape must, beer wort, and dextrose. No surface growth. No fermentation in lactose and saccharose.

*Rates of Fermentation of Torulas 37 and 77 in Various Media.*

Rates of fermentation in grape must of 23° Balling, and 29.8° Balling, beer wort of 14° Balling, and 15% solutions of dextrose, saccharose, and lactose were tested as previously described for apiculatus and mycoderma forms. The results are given in table 5 and figure 10.

TABLE 5

FERMENTATION TESTS AT 33° C OF TORULA FORMS FROM CALIFORNIA YFASTS

Yeast number	Time in hours	Must of 23° Balling, loss in grams	Must of 29°8 Balling, loss, in grams	Wort loss, in grams 14 per cent solution	Dextrose loss in grams 15 per cent solution	Saccharose loss, in grams 15 per cent solution	Lactose loss, in grams 15 per cent solution
37	18	.01	.00	.04	.12	.07	.02
37	42	.19	.07	.31	.54	.29	.07
37	66	.51	.....	.45	.98	.38	.....
37	92	.63	.....	.50	1.40	.43	.08
37	165	.87	.....	.51	2.74	.54	.08
37	236	.87	.13	.51	3.64	.54	.09
77	46	.17	.01	.23	.23	.01	.03
77	73	.84	.15	.48	.48	.....	.....
77	96	1.14	.42	.58	.58	.10	.....
77	118	1.30	.81	.68	.68	.....	.....
77	167	1.37	.86	.78	.78	.10	.....
77	215	1.44	1.03	.83	.83	.....	.....
77	239	1.48	1.08	.95	.95	.....	.03

Torulas 37 and 77 were able to produce only very feeble fermentations in the liquids tested. Torula yeast 77 was not able to ferment must of 30° Balling, or saccharose solution. In must of 23° Balling, and in beer wort the fermentations of the two yeasts were about equal. In must, beer wort, and saccharose the fermentations were completed in a short time, about 120 hours. In comparison with the fermentation curves of the *S. ellipsoideus* yeasts (figs. 12 to 15), the yeasts above gave very weak and incomplete fermentations. The flavors of the fermented liquids were disagreeable. Therefore, the yeasts seem to have no practical value but may cause defective flavors in wine where their growth is not prevented.

*Attenuation and Yields of Alcohol.*—The fermented liquids were analyzed for alcohol with the results reported in table 6.

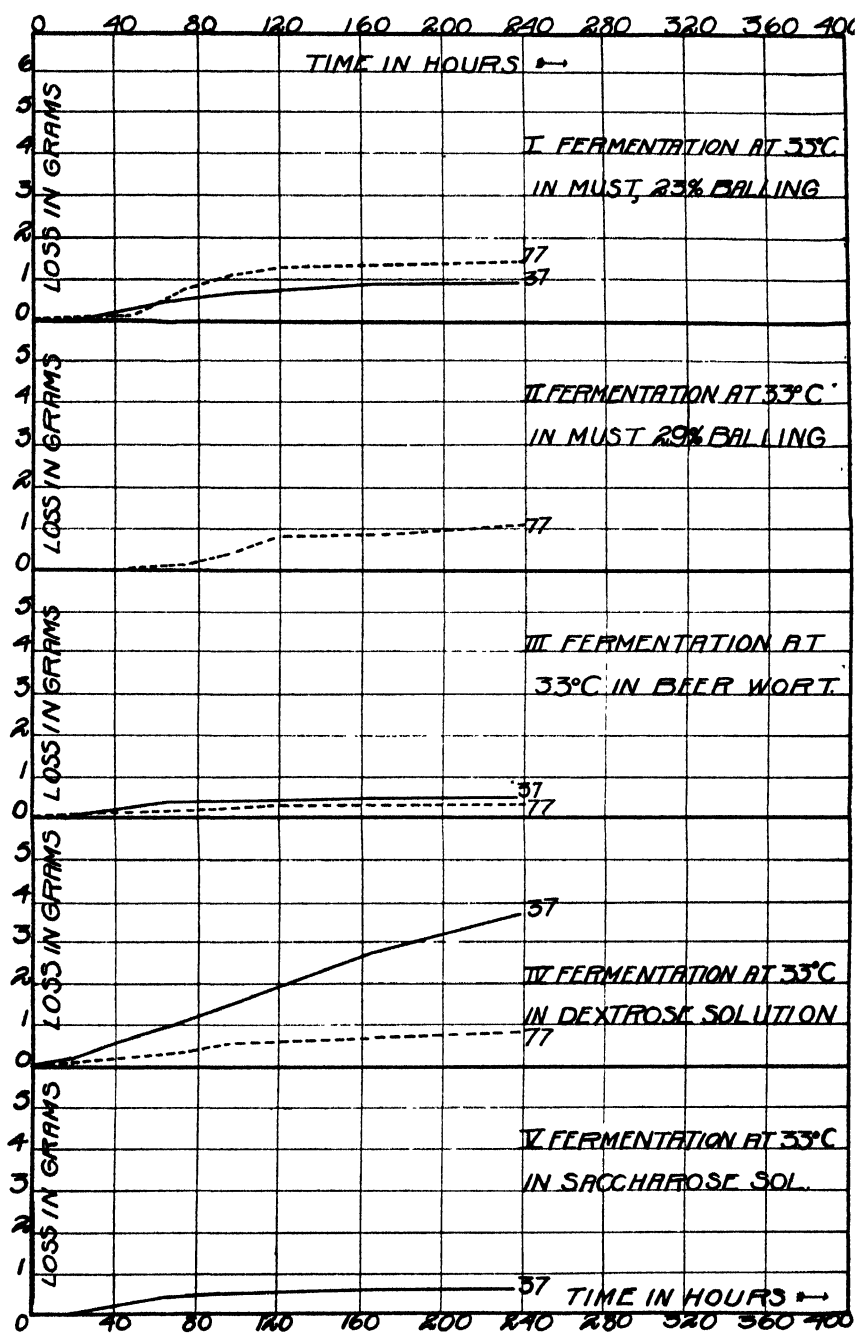


Fig. 10.—Fermentation curves of yeasts 37 and 77 in grape must, beer wort, dextrose and saccharose solutions.

TABLE 6

YEASTS 37 AND 77. ATTENUATION AND YIELDS OF ALCOHOL IN MUST, BEER WORT, DEXTROSE, SACCHAROSE, AND LACTOSE SOLUTIONS

Yeast No.	Medium	Attenuation in grams per 100 c.c.	Theoretical yield of alcohol	Observed yield of alcohol	Per cent of theoretical yield obtained
37	Must of 23° Balling .....	5.21	3.42	.60	17.6
37	Must of 29°8 Balling .....	.05	.03	.00	.0
37	Beer wort of 14° Balling ....	.....	.....	.20	.....
37	Dextrose solution, 15% .....	7.96	5.22	3.50	67.3
37	Saccharose solution, 15% .....	.64	.43	.40	93.02
37	Lactose solution, 15% .....	.06*	.04	.00	.0
77	Must of 23° Balling .....	4.58	2.99	1.31	43.7
77	Must of 29°8 Balling .....	3.85	2.53	.65	25.7
77	Beer wort of 14° Balling ....	2.91	2.00	.30	15.0
77	Dextrose solution, 15% .....	4.55	2.99	.65	21.7
77	Saccharose solution, 15% .....	.....	.....	.00	.0
77	Lactose solution, 15% .....	.06	.04	.00	.0

Analyses of the fermented liquids from fermentation of *Torulas* 37 and 77 in grape must, etc., showed that the absolute amounts of alcohol formed were small and that the yields for each per cent of sugar destroyed were in all cases, except for yeast 37 in dextrose, very low. In the majority of the fermentations, the yields were less than 50 per cent of the theoretical. These yeasts have the defects of giving slow incomplete fermentations, low yields of alcohol for sugar fermented and of producing liquids of disagreeable odor and flavor.

## PASTORIANUS AND WILLIA YEAST FROM CALIFORNIA GRAPES

One *S. pastorianus* and one *Willia* yeast were obtained from grapes in 1911. Both are true yeasts and form spores.

*Organism 36. S. pastorianus Yeast from Grapes Grown at Davis*

*Morphology.*—Microscopical Appearance.—Form varies from spherical to sausage-shaped. The ellipsoidal form predominating. Much smaller than true ellipsoideus yeast (fig. 3).

*Size.*—Average,  $4.5\mu \times 3\mu$ . Maximum,  $7.5\mu \times 3\mu$ . Minimum,  $1.5\mu \times 1.5\mu$ .

*Spore.*—Produced in great abundance in 24 hours on gypsum spore blocks. Spores also form in grape must cultures. Spores spherical in form. Number per cell, 2 to 4. Size of spores,  $2\mu \times 2\mu$  (fig. 7).

*Colonies on Agar Agar.*—Round. Slightly convex. White. Waxy luster. Colonies in agar spherical. Under the low power, the colonies

are surrounded by chains of cells made up of long central cells with pairs of short ellipsoidal cells at the junctures of the larger cells.

Colonies on Gelatin.—Flat. Concentric rings of growth. Outer edges of colonies sharply indented. Gelatin slowly softened but not completely liquefied.

Growth in Liquid Media.—Heavy wrinkled surface growth which sinks to bottom frequently. No visible fermentation. Taste of liquids bitter and disagreeable.

*Organism 69. Willia Species (Saccharomyces anomalus) from Grapes Grown at Acampo, California*

*Morphology.*—Microscopical Appearance.—Size and shape very irregular. The larger cells usually sausage-shaped or ellipsoidal and the smaller ones spherical. Larger than typical *S. anomalus* in the University collection (fig. 3).

Size.—Average,  $6\mu \times 3\mu$ . Maximum,  $14\mu \times 2.5\mu$ . Minimum,  $3\mu \times 3\mu$ .

Spore Formation.—Spores are formed abundantly on gypsum blocks at  $28^{\circ}\text{C}$  and  $22^{\circ}\text{C}$ , in three days. Spores are of typical *Willia* form, i.e., hat-shaped (fig. 7). Number of spores per cell varies from 2 to 8. Size of spores,  $4\mu \times 1.5\mu$ .

Colonies on Agar Agar.—Medium size. Flat. White. Waxy luster. Edges entire. Colonies on the agar are spherical. Under the microscope the edges of the colonies are entire.

Colonies on Gelatin.—Large. Flat. White. Chalklike. Colonies spread rapidly and soon cover entire surface and the gelatin is rapidly liquefied.

Growth in Liquid Media.—A chalky white film develops on all liquids tested, with flocculent growth at bottom of flask. Prolonged top fermentation takes place in all liquids except lactose. Strong odor of ethyl acetate is developed. The fermented liquids possess a burning taste.

*Rates of Fermentation of Yeasts 36 and 69.*—These yeasts were tested for their rates of fermentation in various media as previously described for other organisms. The results are given in table 7 and figure 11.

From the data given in table 7, yeast 36 is seen to give scarcely any fermentation in any of the liquids tested, the maximum loss in weight per 100 c.c. being .68 grams in must of  $29.8^{\circ}$  Balling. On the other hand, yeast 69 gave fairly strong fermentations in must, beer wort, dextrose, and saccharose solutions. Neither yeast fermented lactose,

TABLE 7

DATA OF RATES OF FERMENTATION OF YEASTS NUMBERS 36 AND 69 IN VARIOUS SUGAR SOLUTIONS

Yeast number	Time in hours	Must of 23° Balling, loss in grams	Concentrated must, loss in grams 29°8 Ball.	Wort loss, in grams 14 per cent Balling.	Dextrose loss, in grams 15 per cent solution	Saccharose loss, in grams 15 per cent solution	Lactose loss, in grams 15 per cent solution
36	46	.03	.03	.03	.03	.00	.00
36	73	.04	.04	.03	.06	.00	.00
36	96	.09	.11	.03	.08	.00	.01
36	118	.24	.16	.13	.13	.00	.01
36	167	.33	.43	.13	.19	.00	.02
36	215	.45	.63	.13	.26	.00	.02
36	239	.46	.68	.....	.28	.00	.02
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69	18	.01	.10	.13	.01	.10	.03
69	42	.58	.18	.46	.13	.28	.14
69	66	1.10	.23	.56	.77	.86	.14
69	92	1.79	.80	1.71	1.77	1.90	.....
69	123	2.49	2.20	1.75	2.87	2.88	.....
69	165	3.22	2.50	1.81	3.99	3.78	.....
69	236	4.02	4.88	1.92	5.31	4.62	.14
69	283	4.49	5.55	1.93	5.77	4.98	.....
69	314	4.62	5.75	.....	5.79	5.03	.....

while yeast 36 did not attack saccharose. Although yeast 69 gives more vigorous fermentations than does yeast 36, still it does not approach the *S. ellipsoideus* yeasts from California grapes in rate of fermentation or fermentative power.

*Attenuation and Alcohol Yields of Yeasts 36 and 69.*—The fermented liquids were analyzed with the results given in the accompanying table.

TABLE 8

YEASTS NUMBERS 36 AND 69. ATTENUATION AND YIELDS OF ALCOHOL IN MUST, BEER WORT, DEXTROSE, SACCHAROSE, AND LACTOSE SOLUTIONS

Yeast No.	Medium	Attenuation in grams per 100 c c	Theoretical yield of alcohol	Observed yield of alcohol	Per cent of theoretical yield obtained
36	Must of 23° Balling .....	2.49	1.95	.30	15.3
36	Must of 29°8 Balling .....	1.65	1.08	.00	.0
36	Beer wort of 14° Balling ....	.31	.20	.00	.0
36	Dextrose solution, 15% .....	1.95	1.28	.24	18.9
36	Saccharose solution, 15% .....	.02	.67	.00	.....
36	Lactose solution, 15% .....	.11	.07	.00	.....
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69	Must of 23° Balling .....	11.12	7.30	1.25	17.1
69	Must of 29°8 Balling .....	2.92	1.90	1.00	52.6
69	Beer wort of 14° Balling ....	1.40	.92	.50	54.3
69	Dextrose solution, 15% .....	11.03	7.25	3.10	42.6
69	Saccharose solution, 15% .....	7.73	5.09	2.60	51.2
69	Lactose solution, 15% .....	1.25	.80	.00	.0

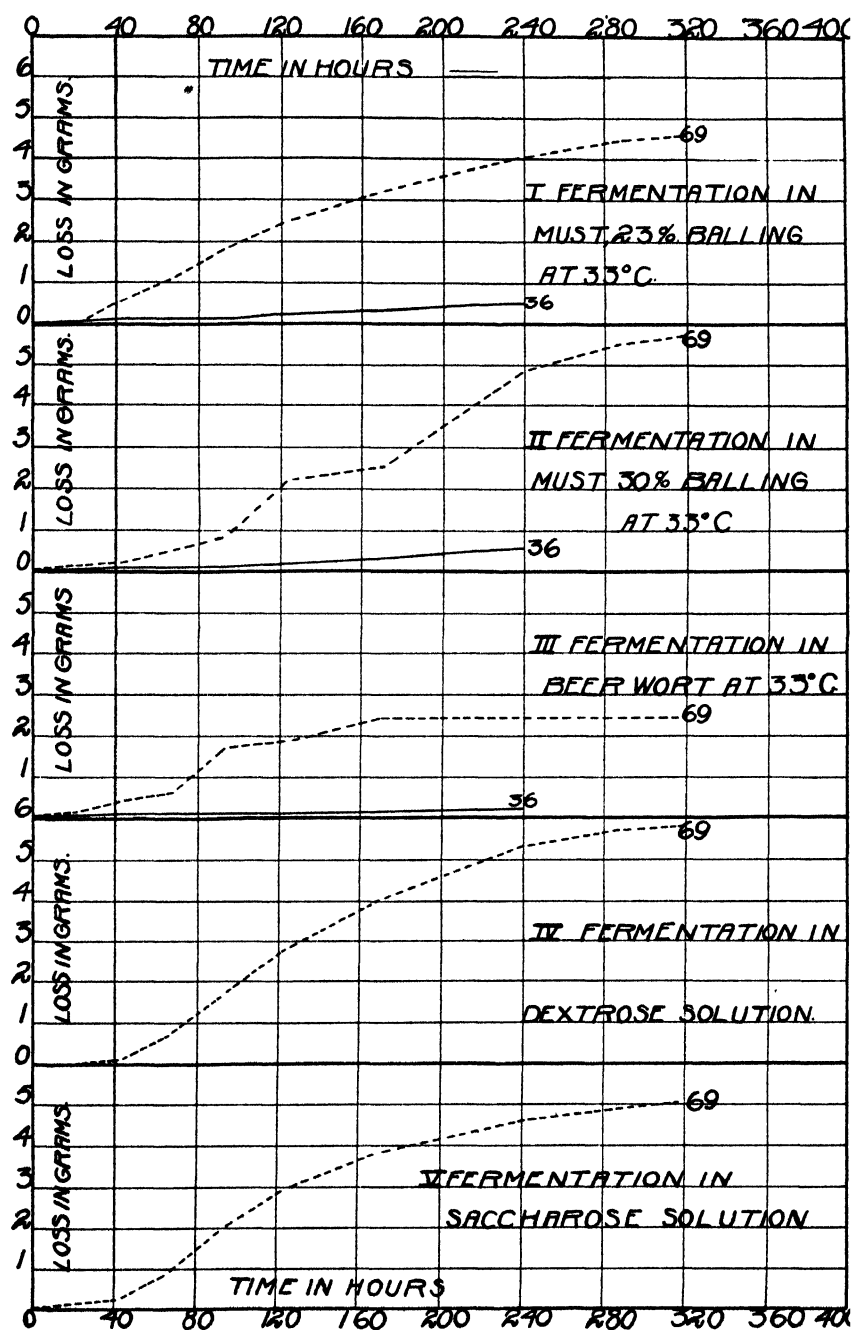


Fig. 11.—Fermentation curves for yeasts 36 and 69 in grape must, beer wort, dextrose, and saccharose solution at 33°C.



Yeast 69 destroyed larger amounts of sugar than did yeast 36 and formed greater amounts of alcohol, but both yeasts gave very low yields of alcohol compared to the amount of sugar fermented. In most cases the yield was below 50% of the theoretical, based on the sugar destroyed. Yeast 69 formed 3.1% alcohol in dextrose solution, this being the largest amount of alcohol produced by either yeast.

Because of its disagreeable flavor and low alcohol formation, yeast 36 is worthless for fermentation purposes. Yeast 69 produces a very aromatic odor and flavor in liquids in which it grows. These fermented materials are not pleasing to drink by themselves but when added in small amounts to new wine they have the property of "aging" the new wine so treated. Therefore it may be possible to use this yeast as an aid to aging wine either by adding some of the liquid fermented by it to new wine or by growing it directly in new wine, and in this latter case, allowing the desired flavors to be developed in the wine itself. Yeasts of this same group are used to some extent in the aging of "sake" (Japanese beer).

#### TRUE WINE YEASTS, *SACCHAROMYCES ELLIPSOIDEUS* FROM CALIFORNIA GRAPES

Six kinds of true wine yeast, *S. ellipsoideus*, were isolated from grapes in 1911 and have been discussed as a group in the following pages.

##### *Organism 64. Saccharomyces ellipsoideus from Grapes* *Grown at Acampo*

*Morphology.*—Microscopical Appearance.—Form varies from shortly ellipsoidal to spherical. Cells shorter than those of no. 74 (fig. 5).

Size.—Average,  $7.5\mu \times 6\mu$ . Maximum,  $12\mu \times 3\mu$ . Minimum,  $3\mu \times 3\mu$ .

Spore Formation.—Abundant spore formation at  $22^{\circ}\text{C}$  and  $28^{\circ}\text{C}$ . Usually 3 spores per cell. Shape spherical. Size,  $3\mu \times 3\mu$ .

Colonies on Agar Agar.—Medium size. Round. Moderately convex. White. Waxy luster. Edges entire. Under microscope edges are entire.

Colonies on Gelatin.—Large. Centers elevated. Radiating ridges from center. Centers slightly elevated. Edges of colonies are undulate in appearance. Gelatin slowly softened but not liquefied. Edges of colonies entire under microscope.

Growth in Liquid Media.—Rapid in grape must, beer wort, dextrose, and saccharose solutions with vigorous fermentation and formation of fine grained sediment. No fermentation in lactose, but fair growth. Characteristic vinous odor and flavor developed.

*Organism 66. Saccharomyces ellipsoideus from Grapes  
Grown at Davis*

*Morphology.*—Microscopical Appearance.—Predominating form oblong with tendency to be pointed at one end. Resembles *S. cerevisiae* in this regard (fig. 5).

*Size.*—Average,  $9\mu \times 5.7\mu$ . Maximum,  $13.5\mu \times 4\mu$ . Minimum,  $4\mu \times 3\mu$ . Considerably larger than most varieties of *S. ellipsoideus* from California grapes.

*Spore Formation.*—Spores are formed abundantly at  $22^{\circ}\text{C}$  and  $28^{\circ}\text{C}$  but more rapidly at the lower temperature. Number per cell 2 to 3. Size,  $3\mu \times 3\mu$ .

*Colonies on Agar Agar.*—Size, medium. Convex. Circular. White. Waxy luster. Edges entire macroscopically and microscopically. Colonies in the agar are usually lens-shaped.

*Colonies on Gelatin.*—Colonies more flat than on agar. Smooth. Under microscope edges of colonies appear bordered with branching chains of ellipsoidal cells. Gelatin slowly softened but not liquefied.

*Growth in Liquid Media.*—Rapid with vigorous fermentation in all solutions tested except lactose. Grows in lactose. Sediment fine grained. Settles quickly after fermentation. Typical vinous flavor developed in fermented liquids.

*Organism 67. Saccharomyces ellipsoideus from Grapes Grown  
in Contra Costa County*

*Morphology.*—Microscopical Appearance.—Ellipsoidal to spherical, former type predominating. Resembles yeast 74 in general appearance (fig. 5).

*Size.*—Average,  $6.75\mu \times 5.25\mu$ . Maximum,  $9\mu \times 6.75\mu$ . Minimum,  $3\mu \times 3\mu$ .

*Spore Formation.*—Large numbers of spores are formed at  $22^{\circ}\text{C}$  and  $28^{\circ}\text{C}$  in 24 hours on spore blocks. Number per cell, 2 or 3. Size,  $3\mu \times 3\mu$ .

*Colonies on Agar Agar.*—Small. Convex. White. Waxy luster. Colonies in agar are spherical. Edges of colonies entire, both macroscopically and microscopically.

Colonies on Gelatin.—Large. Centers of colonies slightly depressed. Edges are undulate. Under microscope, colonies are fringed with short straight chains of ellipsoidal cells. The gelatin is not liquefied or softened.

Growth in Liquid Media.—Growth rapid and fermentation vigorous in grape must, beer wort, dextrose, and saccharose. Growth slight and fermentation absent in lactose. Fermentation more rapid at 20° C than at 33° C. Sediment fine grained. Typical vinous flavor and

*Organism 74. Saccharomyces ellipsoideus from Grapes Grown at Acampo*

*Morphology.*—Microscopical Appearance.—Usual form oblong rather than shortly ellipsoidal. Decidedly different in form than yeast 64 from the same grapes (fig. 5).

Size.—Average,  $6.6\mu \times 4.6\mu$ . Maximum,  $9\mu \times 4.5\mu$ . Minimum,  $3\mu \times 3\mu$ . Smaller than yeasts 66 and 79, which it resembles in shape.

Spore Formation.—Few spores only at 28° C in 48 hours and none in 24 hours. More formed at 22° C in 48 hours. Number per cell, 2. Size,  $3\mu \times 3\mu$  (fig. 6).

Colonies on Agar Agar.—Large. Round. Centers elevated. Colonies in agar lens-shaped. Edges of colonies entire both macroscopically and microscopically.

Colonies on Gelatin.—Large. Low elevation. Colonies in the gelatin spherical. Edges of colonies entire both macroscopically and microscopically. No liquefaction.

Growth in Liquid Media.—Rapid with vigorous fermentation in must, beer wort, dextrose, and saccharose solutions. Small growth and no fermentation in lactose. Typical vinous taste and odor.

*Organism 75. Saccharomyces ellipsoideus from Grapes Grown in Contra Costa County*

*Morphology.*—Microscopical Appearance.—Spherical to shortly ellipsoidal (fig. 5).

Size.—Average,  $6.3\mu \times 5.5\mu$ . Maximum,  $8\mu \times 8\mu$ . Minimum,  $3\mu \times 3\mu$ .

Spore Formation.—No spores formed at 28° C at 72 hours, but abundantly after 144 hours. This differs from yeast 67 from the same grapes; 67 forms spores in 24 hours at 28° C. Numbers of spores per cell, 2. Size,  $3\mu \times 3\mu$  (fig. 6).

Colonies on Agar Agar.—Medium size. Round. Convex. White. Waxy luster. Colonies in the agar are lens-shaped. Edges of colonies entire, both macroscopically and microscopically.

Colonies on Gelatin.—Large. Low and of even elevation. Surface radiately ridged. Edges undulate. Under microscope colonies are bordered by short straight chains of ellipsoidal cells.

Growth in Liquid Media.—Rapid and fermentation vigorous in grape must, beer wort, dextrose, and saccharose. Slight and no fermentation in lactose. Typical vinous flavor and odor in fermented liquids.

*Organism 79. Saccharomyces ellipsoideus from Grapes Grown in Contra Costa County*

*Morphology.*—Microscopical Appearance.—Cells large and oblong. Not strictly ellipsoidal in form (fig. 6).

Size.—Average,  $8.25\mu \times 5\mu$ . Maximum,  $12\mu \times 6\mu$ . Minimum,  $4.5\mu \times 3\mu$ .

Spore Formation.—Spores are formed abundantly in 24 hours at  $28^{\circ}$  C. Number per cell, 2 to 4. Size,  $2\mu \times 2\mu$  and  $3\mu \times 3\mu$  (fig. 6).

Colonies on Agar Agar.—Medium size. Spherical. Convex. White. Waxy luster. Colonies in agar lens-shaped. Edges entire both macroscopically and microscopically.

Colonies on Gelatin. Flat. Edges hazy. White. Waxy luster. Under microscope edges are bordered by branching chains of ellipsoidal cells.

Growth in Liquid Media.—In grape must, beer wort, dextrose, and saccharose, rapid and fermentation vigorous. In lactose, slow and no fermentation. Typical vinous taste and odor developed.

*Fermentation Records at  $33^{\circ}$  C.*—Fermentations were carried out in 100 c.c. portions of various liquids at  $33^{\circ}$  C and records of the rates of fermentation were kept as previously explained.

All of the yeasts with the exception of yeast 67 show rapid fermentations in must of  $23^{\circ}$  Balling, and  $30^{\circ}$  Balling, beer wort, dextrose and saccharose solutions, there being little to choose between them in the rapidity of fermentation and the amount of sugar destroyed. The yeasts show considerable variation in the fermentation of beer wort. Apparently some of the yeasts ferment only the dextrose of the beer wort and not the maltose. Yeast 67, however, is very strongly inhibited in its activity by the moderate temperature of  $33^{\circ}$  C, because it causes only very feeble and incomplete fermentations at this tem-

TABLE 9

DATA ON RATES OF FERMENTATION OF YEASTS NUMBERS 64, 66, 67, 74, 75, AND 79  
AT 33° C IN VARIOUS SUGAR SOLUTIONS

Yeast number	Time in hours	Must of 28° Balling, loss in grams	Must of 29° 8 Balling loss in grams	Wort loss, in grams 14° Ball.	Dextrose loss, in grams 15 per cent solution	Saccharose loss in grams 15 per cent solution	Lactose loss, in grams 15 per cent solution
64	18	1.42	.00	.87	.49	.68	.03
64	42	4.18	.66	2.57	1.95	2.43	.....
64	66	6.72	4.33	2.75	2.32	3.96	.....
64	92	8.09	7.90	2.94	4.74	5.35	.....
64	123	8.26	8.70	2.94	5.84	6.30	.....
64	165	8.33	9.70	3.04	6.49	6.88	.07
64	236	8.88	10.00	3.16	6.77	7.30	.07
66	27	1.83	.05	.06	.44	.60	.00
66	52	5.92	.69	.55	2.13	1.89	.00
66	77	8.49	4.11	2.49	5.23	2.91	.00
66	125	10.09	9.31	6.64	6.81	4.59	.00
66	167	10.44	11.71	8.69	.....	5.56	.00
66	244	10.98	11.85	.....	7.54	7.38	.00
66	341	11.68	12.59	.....	.....	8.35	.00
66	421	.....	12.82	.....	.....	8.55	.00
67	27	.14	.04	.33	.66	.31	.00
67	52	.89	.14	.53	1.80	.42	.00
67	77	1.99	.92	.64	3.21	.64	.00
67	125	2.79	1.57	.67	4.11	.83	.00
67	244	2.99	1.84	.72	5.72	.84	.00
67	341	3.33	2.40	.75	7.07	1.13	.00
67	421	3.50	2.85	.75	7.35	1.30	.00
67	.....	4.59	2.98	.....	.....	1.39	.00
74	27	2.69	.16	1.76	.83	.93	.00
74	52	5.71	2.89	2.54	3.52	2.74	.00
74	77	7.81	6.15	3.99	5.52	4.42	.00
74	125	9.14	9.77	4.27	.....	6.59	.00
74	167	9.31	11.87	4.32	7.23	7.09	.00
74	244	10.87	12.38	4.65	.....	7.62	.00
74	341	11.50	12.53	5.00	.....	7.87	.00
74	421	.....	12.65	5.15	.....	7.97	.00
75	27	2.08	.08	.66	.38	.61	.00
75	52	4.72	.67	1.29	.80	1.57	.00
75	77	6.74	4.05	1.89	2.05	2.47	.00
75	125	8.06	6.89	2.64	3.38	4.04	.00
75	167	8.74	7.94	3.12	6.17	5.17	.00
75	244	9.85	8.88	3.40	6.98	6.78	.00
75	341	10.97	10.66	3.55	7.29	7.60	.00
75	421	11.35	10.66	3.65	.....	7.90	.....
79	46	4.96	3.21	1.81	1.79	1.80	.00
79	73	7.77	6.97	2.42	3.37	3.66	.00
79	96	8.40	8.84	2.63	4.52	4.77	.00
79	118	8.74	10.14	2.83	5.66	5.71	.00
79	167	8.88	10.83	2.83	6.38	6.40	.00
79	215	9.07	11.27	3.07	6.66	6.55	.00
79	239	9.10	11.40	3.07	6.68	6.60	.00

perature. The presence of such yeast as this in natural wine fermentations would render such fermentations very liable to "sticking" if the temperature arose much above  $33^{\circ}\text{C}$ . It is possible that the

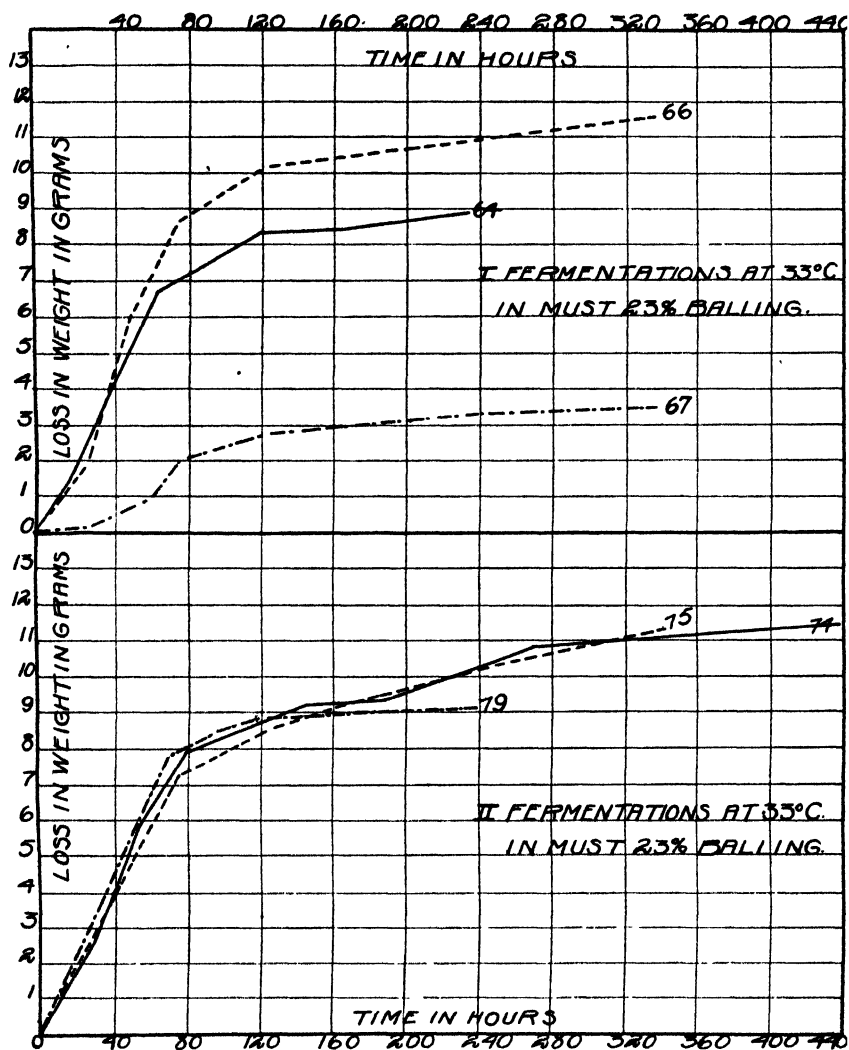


Fig. 12.—Fermentation curves of true wine yeasts from California grapes.

reason that some wines will ferment completely even at temperatures above  $38^{\circ}\text{C}$ , and others stick with unfermented sugar is because of the difference in the character of the natural wine yeast present. The possibility of a yeast similar to yeast 67 being the only wine yeast present in a natural wine fermentation makes it very desirable to

insure good fermentations by the use of pure and selected varieties of wine yeast.

Lactose supported the growth of the *S. ellipsoideus* yeasts discussed above, but was not fermented by any of them.

*Attenuation and Alcohol Formation at 33° C.*—The fermented liquids were analyzed with the results in table 10.

TABLE 10

YEASTS NUMBERS 64, 66, 67, 74, 75, 79. ATTENUATION AND YIELDS OF ALCOHOL IN MUST, BEER WORT, DEXTROSE, SACCHAROSE, AND LACTOSE SOLUTIONS

Yeast No.	Medium	Attenuation in grams per 100 c.c	Theoretical yield of alcohol	Observed yield of alcohol	Per cent of theoretical yield obtained
64	Must of 23° Balling .....	19.70	12.80	11.75	93.4
64	Must of 29°8 Balling .....	21.40	13.90	12.80	92.1
64	Beer wort of 14° Balling .....	6.16	4.39	3.40	77.4
64	Dextrose solution, 15% .....	11.69	7.60	6.00	79.0
64	Saccharose solution, 15% .....	12.23	8.80	7.15	81.4
64	Lactose solution, 15% .....	.....	.....	.00	.0
66	Must of 23° Balling .....	19.41	12.70	11.40	89.7
66	Must of 29°8 Balling .....	19.75	12.90	11.40	88.4
66	Beer wort, of 14° Balling .....	.....	.....	5.70	.....
66	Dextrose solution, 15% .....	13.20	8.60	7.95	92.4
66	Saccharose solution, 15% .....	12.03	8.30	7.55	90.9
66	Lactose solution, 15% .....	.....	.....	.00	.....
67	Must of 23° Balling .....	5.41	3.55	2.00	56.4
67	Must of 29°8 Balling .....	3.85	2.53	1.30	51.3
67	Beer wort of 14° Balling .....	1.61	1.11	.65	58.6
67	Dextrose solution, 15% .....	12.75	8.38	7.95	94.7
67	Saccharose solution, 15% .....	2.82	1.94	1.00	51.5
67	Lactose solution, 15% .....	.36	.24	.00	.0
74	Must of 23° Balling .....	19.31	12.70	11.40	89.7
74	Must of 29°8 Balling .....	20.95	13.70	11.40	83.2
74	Beer wort, of 14° Balling .....	7.96	5.30	4.10	75.3
74	Dextrose solution, 15% .....	11.64	7.60	5.70	75.0
74	Saccharose solution, 15% .....	11.83	8.18	7.95	97.2
74	Lactose solution, 15% .....	.....	.....	.00	.....
75	Must of 23° Balling .....	18.31	12.03	8.01	66.6
75	Must of 29°8 Balling .....	22.10	14.53	.....	.....
75	Beer wort of 14° Balling .....	6.07	4.17	2.34	57.3
75	Dextrose solution, 15% .....	12.55	8.60	6.40	74.4
75	Saccharose solution, 15% .....	12.62	8.70	8.60	98.8
75	Lactose solution, 15% .....	.36	.25	.00	.0
79	Must of 23° Balling .....	18.51	12.17	.....	.....
79	Must of 29°8 Balling .....	18.54	12.20	11.45	93.8
79	Beer wort of 14° Balling .....	6.36	4.38	2.35	53.7
79	Dextrose solution, 15% .....	12.60	8.20	6.00	72.9
79	Saccharose solution, 15% .....	11.43	7.87	6.00	76.2
79	Lactose solution, 15% .....	.06	.04	.00	.0

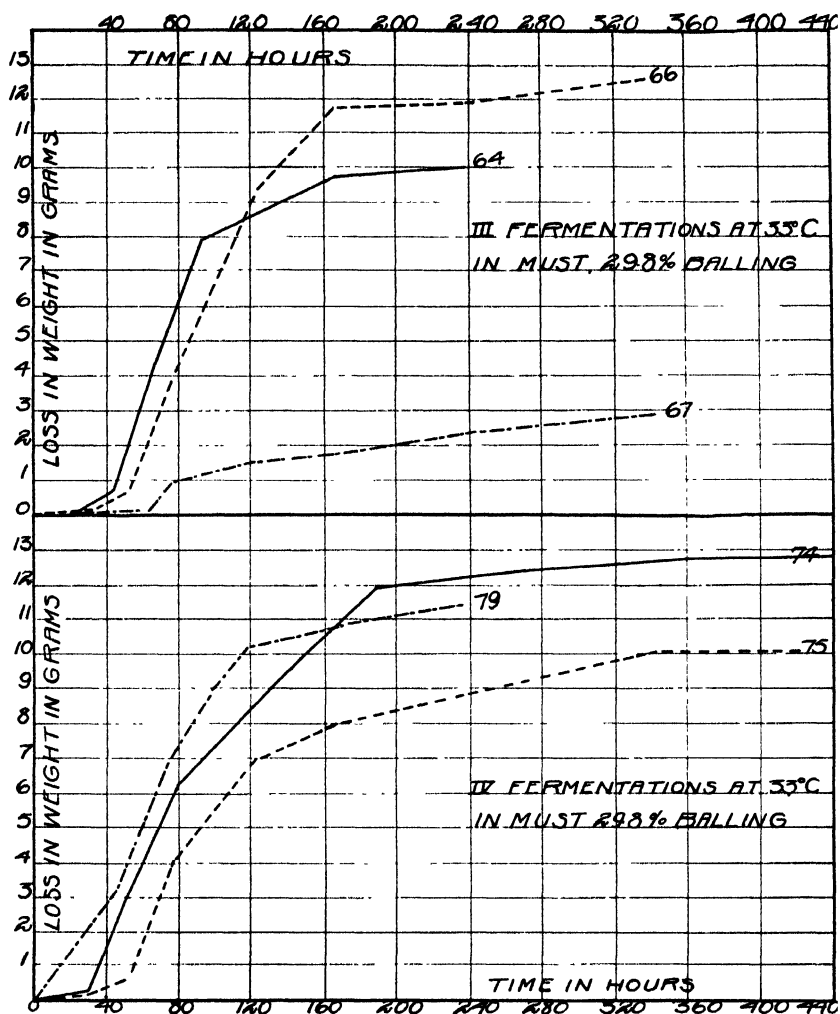


Fig. 13.—Fermentation curves of true wine yeasts from California grapes.

The effect of a moderately high temperature on yeast 67 is shown by the analyses of the fermented liquids, from which it is seen that the absolute amounts of alcohol formed by this yeast are small and that the per cents of the theoretical yields obtained were very small. Thus at 33° C it not only gives very incomplete fermentations, but is also very wasteful of the sugar that it ferments. Yeast 75 gave the highest yield of alcohol, 14.53 per cent. None of the yeasts gave as much alcohol in must of 30° Balling, at 33° C as they did at 24° C. Contrasted with the yeasts discussed in the previous pages the *S. ellipsoideus* yeasts gave larger yields of alcohol and in general a larger per cent of the sugar destroyed was transformed into alcohol.



## COMPARISON OF FERMENTATIONS AT 33° C AND 24° C (ALL VARIETIES)

The nineteen fermentation organisms discussed in the preceding pages were transferred to grape must of 29.8° Balling, and the products of fermentation at 24° C and 33° C studied and compared. The results appear in table 11.

TABLE 11

COMPARISON OF PRODUCTS OF FERMENTATION OF CALIFORNIA YEASTS IN GRAPE MUST OF 30° BALLING FERMENTED AT 24° C AND 33° C

Organism	Attenuation in grams per 100 c.c.	Theoretical yield of alcohol, volume, per cent	Alcohol obtained, volume, per cent	Per cent of theoretical yield obtained	Temperature
35. <i>S. apiculatus</i> .....	8.31	5.46	2.60	47.6	24° C
72. <i>S. apiculatus</i> .....	6.80	4.47	3.20	72.7	24° C
65. <i>Mycoderma</i> species ....	2.70	1.70	0.00	00.0	24° C
68. <i>Mycoderma</i> species ....	4.20	2.70	1.20	44.4	24° C
70. <i>Mycoderma</i> species ....	3.40	2.20	.60	27.3	24° C
71. <i>Mycoderma</i> species ....	8.20	5.40	3.20	59.2	24° C
73. <i>Mycoderma</i> species ....	23.90	15.70	13.50	86.0	24° C
76. <i>Mycoderma</i> species ....	3.20	2.10	.65	30.9	24° C
78. <i>Mycoderma</i> species ....	10.10	6.64	4.05	60.9	24° C
37. <i>Torula</i> species .....	3.70	2.43	.30	12.3	24° C
77. <i>Torula</i> species .....	6.47	4.25	1.05	24.7	24° C
36. <i>S. pastorianus</i> .....	2.80	1.85	0.00	00.0	24° C
69. <i>Willia anomala</i> .....	5.11	3.35	1.05	31.3	24° C
64. <i>S. ellipsoideus</i> .....	24.60	16.20	15.60	96.3	24° C
66. <i>S. ellipsoideus</i> .....	22.00	14.40	14.10	97.9	24° C
67. <i>S. ellipsoideus</i> .....	24.01	15.80	15.30	96.8	24° C
74. <i>S. ellipsoideus</i> .....	22.50	14.80	14.20	95.2	24° C
75. <i>S. ellipsoideus</i> .....	22.70	14.90	14.40	96.6	24° C
79. <i>S. ellipsoideus</i> .....	25.30	16.60	15.55	93.7	24° C
<hr/>					
35. <i>S. apiculatus</i> .....	6.15	.79	.30	37.9	33° C
72. <i>S. apiculatus</i> .....	1.20	4.03	2.35	58.30	33° C
65. <i>Mycoderma vini</i> .....	0.00	0.00	0.00	00.0	33° C
68. <i>Mycoderma vini</i> .....	7.84	5.15	4.50	89.30	33° C
70. <i>Mycoderma vini</i> .....	2.05	1.35	1.00	74.50	33° C
71. <i>Mycoderma vini</i> .....	.....	.....	7.90	.....	33° C
73. <i>Mycoderma vini</i> .....	14.75	9.70	6.80	70.10	33° C
76. <i>Mycoderma vini</i> .....	00.00	0.00	0.00	00.00	33° C
78. <i>Mycoderma vini</i> .....	14.13	9.29	9.00	97.90	33° C
37. <i>Torula</i> species .....	.05	.03	0.00	00.00	33° C
77. <i>Torula</i> species .....	3.85	2.53	.65	25.70	33° C
36. <i>S. pastorianus</i> .....	1.65	1.08	.00	00.00	33° C
69. <i>Willia Anomala</i> .....	2.92	1.90	1.00	52.60	33° C
64. <i>S. ellipsoideus</i> .....	21.40	13.90	12.80	92.10	33° C
66. <i>S. ellipsoideus</i> .....	19.75	12.90	11.40	88.40	33° C
67. <i>S. ellipsoideus</i> .....	3.85	2.53	1.30	51.30	33° C
74. <i>S. ellipsoideus</i> .....	20.95	13.70	11.40	83.20	33° C
75. <i>S. ellipsoideus</i> .....	22.10	14.53	.....	.....	33° C
79. <i>S. ellipsoideus</i> .....	18.54	12.20	11.45	93.80	33° C

TABLE 11—(Continued)

Organism	Volatile acid, grams per 100 c.c.	Loss in fixed acid during fermentation, grams per 100 c.c.	Temperature
35. <i>S. apiculatus</i> .....	.110	— .10	24° C
77. <i>S. apiculatus</i> .....	.100	.05	24° C
65. <i>Mycoderma vini</i> .....	.020	.06	24° C
68. <i>Mycoderma vini</i> .....	.050	— .19	24° C
70. <i>Mycoderma vini</i> .....	.030	— .10	24° C
71. <i>Mycoderma vini</i> .....	.043	— .07	24° C
73. <i>Mycoderma vini</i> .....	.097	.02	24° C
76. <i>Mycoderma vini</i> .....	.020	.04	24° C
78. <i>Mycoderma vini</i> .....	.056	— .08	24° C
37. <i>Torula species</i> .....	.084	.06	24° C
77. <i>Torula species</i> .....	.020	— .02	24° C
36. <i>S. pastorianus</i> .....	.030	— .04	24° C
69. <i>Willia anomala</i> .....	.080	— .06	24° C
64. <i>S. ellipsoideus</i> .....	.060	.06	24° C
66. <i>S. ellipsoideus</i> .....	.050	.10	24° C
67. <i>S. ellipsoideus</i> .....	.080	.12	24° C
74. <i>S. ellipsoideus</i> .....	.078	.11	24° C
75. <i>S. ellipsoideus</i> .....	.070	.06	24° C
79. <i>S. ellipsoideus</i> .....	.090	.02	24° C

*Discussion of Results in Table 11.*—Organisms 35, 72, 73, 76, 37, 64, 66, 67, and 74 gave higher percentages of the theoretical yield at 24° C than at 33° C. Organisms 68, 70, 77, and 69 gave higher percentages of the theoretical at 33° C than at 24° C and organism 79 gave approximately the same percentages at 33° C and 24° C, while organisms 65 and 36 gave no alcohol at either temperature.

In regard to the absolute amounts of alcohol formed, the relations are somewhat different. Yeasts 68, 70, 71, and 78 gave higher yields of alcohol at 33° C than at 24° C. All of these are *Mycoderma* forms. This might indicate that these forms have a higher optimum than the other forms tested. All other forms tested gave higher yields of alcohol at 24° C than at 33° C. The results are especially striking with *S. ellipsoideus*, number 67, in this respect. It gave 1.3% alcohol at 33° C and 15.3% at 24° C.

The wild yeasts, *i.e.*, the *S. apiculatus*, *Mycoderma*, *Torula*, *S. pastorianus*, and *Willia* yeasts, with the exception of organism 73, all gave small amounts of alcohol and were very wasteful of the sugar fermented. The percentage of the theoretical yield varied from 0% to 72%. On the other hand, the *S. ellipsoideus* yeasts gave fairly high amounts of alcohol, varying from 14.1% to 15.6% at 24° C, and all gave over 93% of the theoretical yield of alcohol from the sugar destroyed at 24° C. These facts emphasize the necessity of favoring

the growth of the true wine yeasts and discouraging the development of the wild yeasts.

It is commonly held by enologists in general that wild yeasts as a rule destroy large amounts of tartaric acid and form high amounts of volatile acid during fermentation. To test this belief with the California wild yeasts under observation, analyses of the original unfermented must used in the above tests and of the fermented liquids were made. Contrary to expectations they did not destroy abnormally large amounts of fixed acid during fermentation, so that it is possible that the low yield of fixed acid in naturally fermented wines compared to those fermented with pure yeast and sulfurous acid may be due to other causes than the development of the wild yeasts. For example, it may be due to bacteria, or the higher yield of fixed acid in wines in which sulfurous acid has been used may be due to the chemical effect of the sulfurous acid on the cream of tartar which it may protect from precipitation by the alcohol of the wine. The fact that the use of sulfurous acid in wine making gives a higher yield of fixed acid in the finished wine made from the same grapes by natural fermentation, and the results of the above tests, simply cast doubt on the theory held that the low yield of fixed acid in naturally fermented wines is due to the activity of the wild yeasts. They do not necessarily prove the contrary view, because of the small number of yeasts under observation.

The largest amount of volatile acid was formed by the *S. apiculatus* yeast, which gave 0.11%. This, in itself, is considerably below the legal limit of 0.140% for red wines and 0.120% for white wines. The real point to note, however, is that the *apiculatus* yeast develops in natural wine fermentations before the true wine yeast has had a chance to carry on any great amount of fermentation, so that the volatile acid formed by the *apiculatus* yeast is added to that later formed by the true wine yeast and other yeasts. In this way the volatile acid may rise dangerously high. Added to this, is the fact that the *apiculatus* yeast produces compounds during fermentation that are prejudicial to the activity of the true wine yeast and so cause it to behave abnormally and produce larger amounts of volatile acid than it otherwise would. It may be stated that wine ceases to be commercially salable when it contains more than 0.14% volatile acid.

From the data and discussions given in the foregoing pages on the properties of California yeasts, it may be said that the yeasts of the *S. ellipsoideus* or true wine yeast type are the only ones so far found

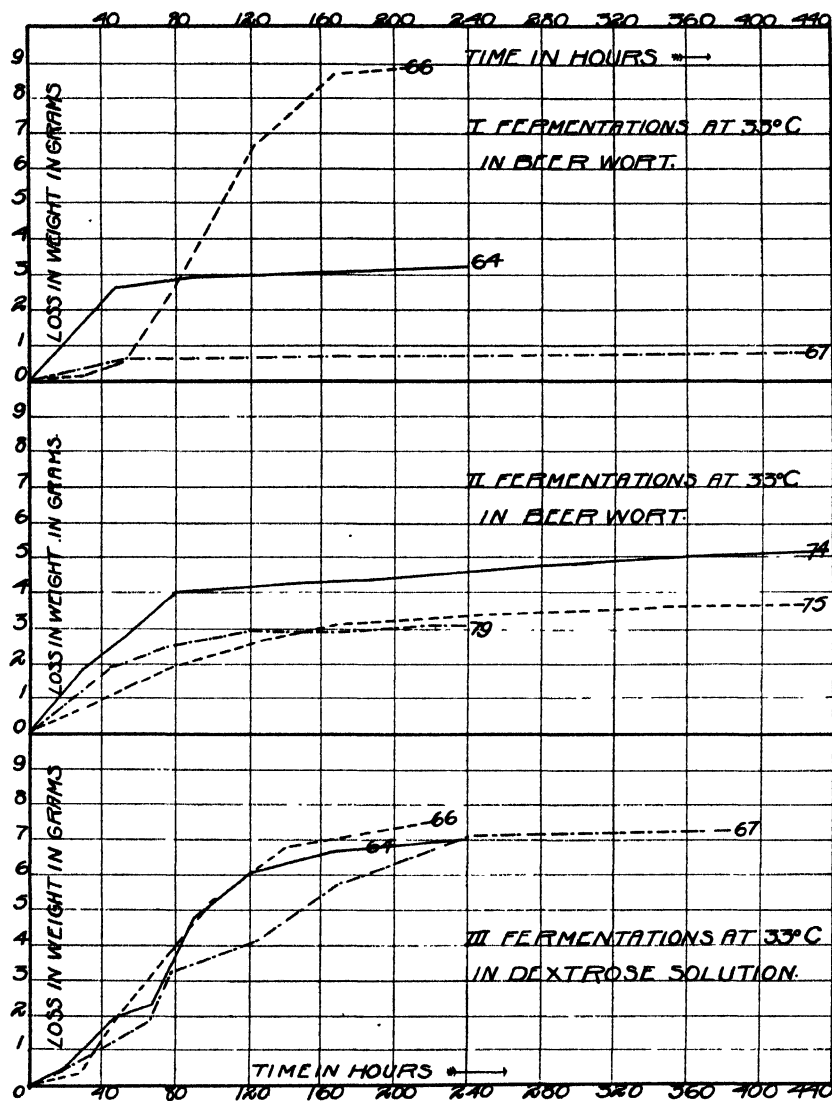


Fig. 14.—Fermentation curves of true wine yeasts from California grapes.

that are suitable for the production of wine. Although several of the wild yeasts produce fairly large amounts of alcohol and low volatile acid during fermentation, they can not be used in wine making, because they produce undesirable flavors and odors. An objection to the California *S. ellipsoideus* yeasts is the fine grained character of their sediments, giving slow clearing after fermentation and difficulty in racking. Another defect is that some of them seem to be weakened very perceptibly by such a moderate temperature as 90° F (32° C).

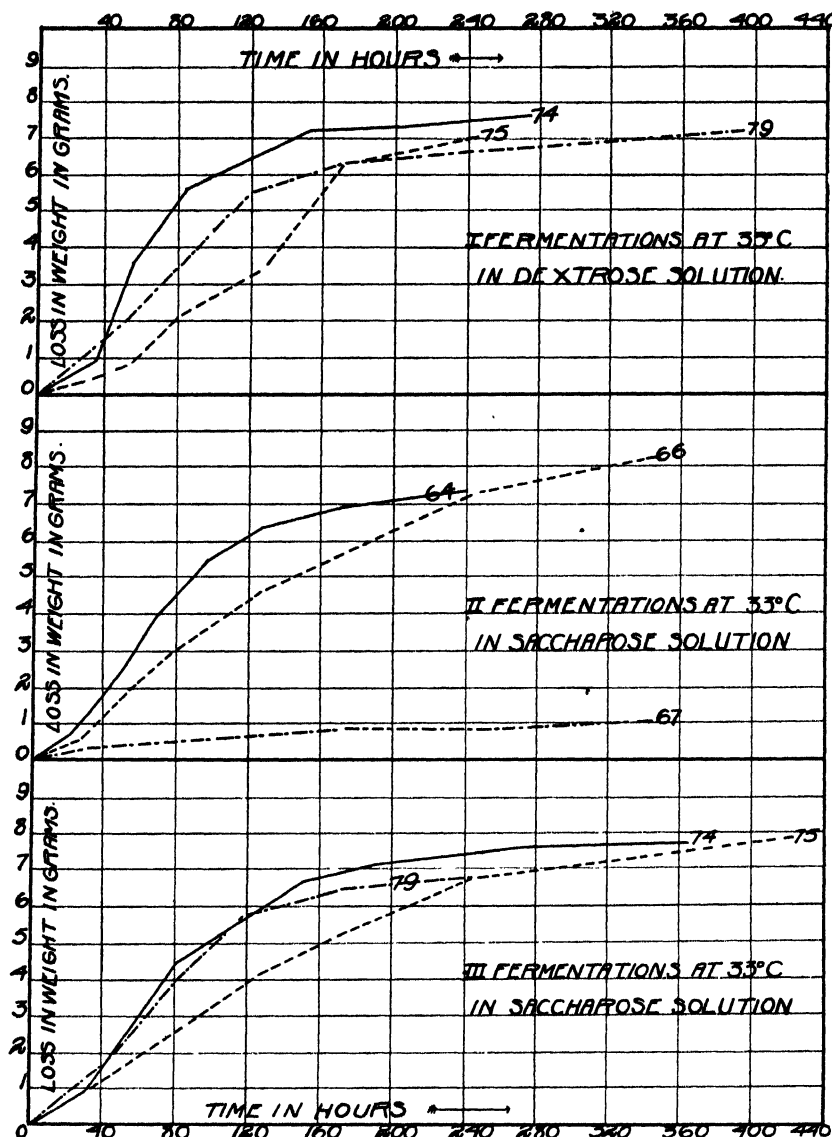


Fig. 15.—Fermentation curves of true wine yeasts from California grapes

Several varieties of *S. ellipsoideus* isolated in France and cultures of which are in the University collection give much better results than any of the yeasts isolated from California grapes in this laboratory. Consequently it seems desirable to use one of the French varieties rather than any of the above California yeasts for commercial fermentation of grapes for wine.

#### IV. INFLUENCE OF LOCALITY ON THE CHARACTER OF THE MICRO-ORGANISMS ON GRAPES

##### METHODS OF TAKING SAMPLES<sup>a</sup>

Samples of grapes were obtained from Davis, Fresno, and El Centro, all localities in California. Two Mason fruit jars of one quart capacity each were plugged with cotton wool, packed into a small box with excelsior, and the box and contents were sterilized at 150° C in a dry-heat sterilizer. Two such jars were sent to each of the above localities. Several small bunches of grapes were cut and allowed to fall into the open jars. The cotton plugs were then replaced. The grapes were sent to Berkeley where the numbers of the different groups of yeasts present were determined. Samples from Contra Costa County, California, were taken in sterile paper bags. The organisms on grapes from Ripon, San Joaquin County, California, were determined at the vineyard.

##### METHOD OF COUNTING LIVING CELLS ON GRAPES

The same sort of grape must agar, and sterile petri dishes previously described were employed in the counting tests. In addition to the above apparatus a number of sterile one c.c. pipettes, and test tubes, each containing 9 c.c. of sterile water were necessary. On arrival at the laboratory, the grapes were crushed in the jars by means of a sterile pestle. One cubic centimeter of the fresh juice was removed by means of a sterile 1 c.c. pipette and transferred to a tube of 9 c.c. sterile water. With another sterile 1 c.c. pipette one cubic centimeter of the liquid in the first tube of water was transferred to a second tube. This process was carried progressively to a third, fourth, and occasionally a fifth tube, depending on the condition of the grapes. This gave a dilution of 1:10 in the first tube, 1:100 in the second tube, 1:1000 in the third, and 1:10,000 in the fourth. From each of these tubes one cubic centimeter of the liquid was removed to separate petri dishes. Melted agar must at 40° C to 45° C was poured into each plate and the plates set away to allow development of the individual cells into colonies. Counts of the number of colonies and notes of the numbers of each type of organism present were made. From the dilutions used it was possible to calculate the number of active cells of each kind of micro-organism present in a unit volume of the original liquid (see plate 1).

<sup>a</sup> The writer wishes to express his appreciation of the aid given him by Messrs. Flossfeder, Way, and Packard in taking samples.

## RESULTS OF COUNTING

The results of the countings made, appear in the following tables:

TABLE 12  
MICRO-ORGANISMS ON GRAPES FROM DAVIS

Organism	Sample number 1, first crop, Muscat grapes, cells per c.c.	Sample number 2, second crop, Muscat grapes, cells per c.c.
Penicillium, green mold (probably glaucum)	none	none
Aspergillus mold (probably <i>niger</i> )	none	1,000
Mucor mold	20,000	none
Dematium mold	130,000	105,000
Powdery white mold	none	3,000
<i>S. apiculatus</i> (a wild yeast)	less than 100*	less than 100
Other wild yeasts	200,000	.....
True wine yeast ( <i>S. ellipsoideus</i> )	none	none

\* The dilutions below 1:100 were so badly overgrown with the dematium mold that it was not possible to differentiate the yeasts present, so that "less than 100 per c.c." may mean from none to 100 per c.c. It signifies that no *S. apiculatus* or true wine yeasts appeared on the dilution of 1:100. There were no true wine yeast cells present, however, because all of the samples were plated out again during fermentation and in this way true wine yeast cells were proven to be absent. Added proof of the absence of true wine yeast is given by the fact that the crushed grapes did not undergo true wine yeast fermentation but after a feeble wild yeast fermentation became moldy and rotted.

TABLE 13  
MICRO-ORGANISMS ON GRAPES FROM EL CENTRO

Organism	Sample number 1, cells per c.c.	Sample number 2, cells per c.c.
Penicillium (olive green mold)	2,400,000	20,000,000
Penicillium (blue green mold)	none	50,000
Fusarium-like mold	30,000	none
Aspergillus mold	40,000	100,000
<i>S. apiculatus</i>	less than 100	less than 100
Wild yeasts, <i>Mycoderma vini</i> type	400,000	8,000,000
True wine yeasts ( <i>S. ellipsoideus</i> )	none	none

There were no true wine yeasts present because the grapes did not undergo a wine yeast fermentation but simply became very moldy and finally rotted.

TABLE 14  
MICRO-ORGANISMS FROM GRAPES GROWN AT FRESNO

Organism	White grapes, cells per c.c.	Red grapes, cells per c.c.
Penicillium (olive green mold)	10,000	15,000
Aspergillus mold	10,000	1,000
Fusarium-like mold	10,000	4,000
Dematium mold	7,500	560,000
Mucor mold	200	less than 100
<i>S. apiculatus</i>	less than 100	less than 100
True wine yeast ( <i>S. ellipsoideus</i> )	less than 100	less than 100

These grapes underwent true wine yeast fermentation, proving the presence of a few *S. ellipsoideus*.

TABLE 15

MICRO-ORGANISMS ON GRAPES FROM VINEYARD OF J. SWETT &amp; SON, MARTINEZ

Organism	Alicante Bouschet grapes, verte grapes, cells per c.c.	Sauvignon cells per c.c.	Isabella grapes, cells per c.c.
Penicillium (green mold) .....	5,000	3,000	15
Mucor (gray mold) .....	2,000	none	none
Dematium (tree mold) .....	17,000	22,000	18,000
Aspergillus mold .....	none	none	200
Monilia mold .....	12,000	30,000	10,000
Wild yeasts .....	14,000	36,000	100
True wine yeast ( <i>S. ellipsoideus</i> ) .....	a few	a few	a few

These grapes underwent normal alcoholic fermentation, proving the presence of *S. ellipsoideus*.

TABLE 16

MICRO-ORGANISMS FROM GRAPES GROWN AT RIPON\*

Organism	Cells per c.c.
Penicillium (blue green mold) .....	1,700
Mucor mold .....	100
Aspergillus mold .....	less than 100
Wild yeasts .....	2,600
True wine yeasts .....	less than 100 but more than 0

\* The above counts were made by plating the grapes in the vineyard.

## YEASTS FROM THE TULARE EXPERIMENT STATION

In 1906 grapes were gathered at the Tulare substation by Professor Bioletti<sup>7</sup> under conditions that precluded contamination by organisms not present on the grapes. The yeasts present on them were isolated by H. C. Holm and their properties studied. All of them were found to be wild yeasts of very low fermenting power and all gave undrinkable fermented musts. No true wine yeast was found.

## DISCUSSION OF TABLES 12 TO 16, INCLUSIVE

The grapes from Davis, Fresno, El Centro, Ripon, and Martinez were all allowed to ferment after crushing and plating tests were made during fermentation in order to obtain the true wine yeast if present. The Davis grapes and the El Centro samples did not undergo true yeast fermentations, but after a short wild-yeast fermentation, became very moldy and finally rotted. The grapes from the other localities underwent normal true wine yeast fermentations and the yeasts of this type present were found on dilutions in agar must made during fermentation.

<sup>7</sup> Bioletti, F. T., and Holm, H. C., Calif. Exp. Sta. Bull. 197, pp. 169, 175, 1908



Wine is not made in the regions in the immediate vicinity of Tulare and El Centro, and none is made at the University Farm, Davis. The grapes from Fresno, Ripon, and Martinez came from places near wineries and on all of these samples true wine yeasts were found. These tests indicate that grapes from regions remote from wineries have smaller numbers of true wine yeast cells on their surfaces than grapes from wine making regions. Reasoning from these facts it is probable that the grapes gathered during the beginning of the season will have fewer true wine yeast cells than those picked later in the crushing season. Thus the use of pure yeast is more necessary on grapes from regions distant from wineries and on grapes gathered during the forepart of the season than it is on those picked in wine making regions after the season has progressed for several weeks. The figures given in Part IV indicate the influence of the time of picking on the numbers of yeasts, etc., present. .

All the samples tested above gave a great preponderance of molds and wild yeasts over the true wine yeasts. It can easily be seen that if there are several hundred thousand wild yeasts and a million or two mold cells for each one to a hundred true yeast cells, as was actually the case in several instances, the true wine yeasts will have overwhelming odds against which to develop. Where such a condition of affairs exists the wild yeasts must of necessity cause considerable damage. It is not probable that the molds do a great deal of damage after crushing because the conditions are not very favorable for their growth in the crushed grapes, but they undoubtedly depreciate the value of grapes for wine making during long shipments.

In summarizing the above data it may be stated that grapes, both from wine making regions and from those in which wine is not made, are deficient in true wine yeasts and carry a large number of molds and wild yeasts prejudicial to the making of the best wines. Grapes from regions remote from wineries seem to bear smaller numbers of the true wine yeasts than those from wine making localities.

## V. INFLUENCE OF THE STAGE OF RIPENESS ON THE CHARACTER OF THE MICRO-ORGANISMS ON GRAPES

### SAMPLING

Grapes were gathered in sterile paper bags from a Zinfandel vine in a large vineyard at Muir Station in Contra Costa County, California, in 1912. One sample was taken when the grapes were green, a second when they had begun to turn, and the third in the middle of

September when the grapes were ripe. Since the samples were all from the same vine, they may be taken as being more or less comparative. A sample was taken from an Alicante Bouschet vine in the same vineyard as the Zinfandel when the grapes were green and a second sample in the middle of September from another vine of the same variety in the vicinity of the first one.

The grapes were crushed in the laboratory, using precautions against outside infection, and counts of the active cells present were made as in the tests recorded in Part III.

## RESULTS

The results of the tests appear in the accompanying tables.

TABLE 17

NUMBERS OF MICRO-ORGANISMS ON GRAPES AT DIFFERENT STAGES OF RIPENESS

Ripeness of grapes at sampling	Type of organisms found	Cells per c.c. of must, Zinfandel grapes	Cells per c.c. of must, Alicante Bouschet grapes
Hard, green	Molds	1,040,000	100
	Wild yeasts	less than 10	less than 10
	<i>S. ellipsoideus</i>	none	none
Beginning to color	Molds	1,000,000	
	Wild yeasts	175,000	
	<i>S. ellipsoideus</i>	none	
Ripe	Molds	190	22,000
	Wild yeasts	3,360	26,000
	<i>S. ellipsoideus</i>	less than 1, but present	a few

The crushed samples were allowed to stand in sterile jars plugged with cotton. Samples taken when the grapes were hard green and beginning to color, molded and putrefied, but did not ferment, indicating absence of *S. ellipsoideus*. The samples of ripe grapes both fermented, showing presence of *S. ellipsoideus*.

On the green grapes molds were about the only kind of organisms present. When the grapes began to turn in color the wild yeasts had made their appearance in large numbers, but no true yeasts were in evidence. On the ripe grapes were found chiefly wild yeasts and molds and in addition to these a few *S. ellipsoideus* cells, not enough to develop on the plates of dilution 1:10, but still enough to cause the grapes to undergo a true yeast fermentation after standing several days in a sterile flask plugged with cotton wool.

The surfaces of the hard green grapes are very poor places for the development of micro-organisms; and molds predominate probably because they are merely present in the dust on the surface of the grapes. As the grapes soften during ripening some of them are broken by birds and insects and the yeasts, etc., develop in the broken berries. From these the cells are carried to the surfaces of other grapes by insects, etc., so that as the season advances the numbers of yeasts increase. Even on the ripe grapes, however, the numbers of the true wine yeast cells, *S. ellipsoideus*, were small and were greatly exceeded by those of the molds and wild yeasts. The data of this table do not confirm the statement often made that the numbers of micro-organisms increase during ripening.

## VI. CHANGES IN THE NUMBERS AND CHARACTER OF THE MICRO-ORGANISMS ON GRAPES DURING SHIPMENT FROM VINEYARD TO CELLAR

Burger grapes were picked at the vineyard of J. Swett & Son, Ripon, California, and dilution plates on agar were made at the vineyard on the freshly picked grapes. Counts were also made on grapes from the same block of the vineyard after they had stood about eighteen hours in boxes. The sample in this latter case was taken from a lot of two boxes that were crushed into a sterile barrel. After the grapes arrived at the winery in Martinez three days later an average sample was taken and counts of the micro-organisms present again made.

Alicante Bouschet grapes were plated at the vineyard and on arrival three days later at the winery. The results of the above tests appear in tables 18 and 19.

TABLE 18  
CHANGE IN MICRO-ORGANISMS ON BURGER GRAPES AFTER PICKING

Organism	Grapes in vineyard, cells per c.c. of must	Grapes after 18 hours in boxes, cells per c.c. of must	Grapes after 3 days on board cars, cells per c.c. of must
1. <i>Penicillium</i> mold .....	1,700	145,000	} 9,200,000
2. <i>Mucor</i> mold .....	100	50,000	
3. <i>Aspergillus</i> mold .....	none	none	
4. <i>Dematium</i> mold .....	less than 1	72,000	
5. <i>Mycodera</i> forms and <i>Torula</i> yeasts .....	2,500	160,000	4,700,000
6. <i>S. apiculatus</i> yeast .....	less than 1	less than 10	less than 1,000
7. True wine yeast ( <i>S. ellipsoideus</i> ) .....	less than 1	less than 100	500,000
8. Vinegar bacteria .....	none	none	800,000

TABLE 19

## CHANGE IN MICRO-ORGANISMS ON ALICANTE BOUSCHET GRAPES DURING SHIPMENT

Organism	Grapes in vineyard, cells per c.c. of must	Grapes three days later at cellar, cells per c.c. of must
1. <i>Penicillium</i> mold .....	320,000	2,500,000
2. <i>Mycoderma</i> forms .....	125	5,400,000
3. Other wild yeasts .....	none	less than 1,000
4. True wine yeast ( <i>S. ellipsoideus</i> ).....	25	440,000
5. Vinegar bacteria .....	none	less than 100

The effect of standing over night in the boxes was to greatly increase the numbers of wild yeasts and molds present without materially affecting the quantity of true wine yeast present. No doubt a great deal of the mold and wild yeast cells came from the sides and bottoms of the boxes, where they had collected from broken and moldy berries during previous shipments. Thus, it may be seen how grapes almost free from micro-organisms could acquire large numbers by standing in boxes a few hours. Some of the increase is, of course, due to normal increase of the cells present on the surface of the grapes and to increase of organisms in the grapes broken during picking.

The grapes were three days on the road from the vineyard to the winery and arrived in a moldy condition, but in no worse state than other Burger grapes shipped under like conditions. The plating tests bore out what the appearance indicated, namely, the presence of great numbers of molds, 9,000,000 per cubic centimeter; wild yeasts, 4,700,000, and even large numbers of vinegar bacteria, 800,000 per cubic centimeter. The wine yeast was present in less numbers than any of the other organisms, there being only 500,000 of them per cubic centimeter.

The Alicante Bouschet grapes gave similar results, but being firmer grapes they arrived in better condition than the Burgers, as was shown by their appearance and by counting tests.

A method of controlling the micro-organisms on grapes during shipment is discussed under Part VII.

## VII. CHARACTER AND NUMBERS OF MICRO-ORGANISMS ON GRAPES AS RECEIVED AT THE WINERY DURING THE SEASONS OF 1911 AND 1912

Samples of the freshly crushed grapes from two wineries in Contra Costa County were taken in 1911 and 1912 and counts of the active cells present were made with the results given in the following table:

TABLE 20  
ORGANISMS ON GRAPES AS RECEIVED AT THE CELLAR IN SEASONS 1911 AND 1912

Source and variety of grapes	Mold cells per c.c. of must	Apiculatus yeast cells per c.c. of must	Other wild yeast cells per c.c. of must	Ellipoides Wine yeast cells per c.c. of must	Vinegar bacteria cells per c.c. of must
<b>1911:</b>					
1. Palomino grapes, shipped 75 miles by rail from Acampo .....	97,600	7,341,000	*	58,500	.....
2. Zinfandel from Acampo .....	1,600,000	2,830,000	30,000	20,000	.....
3. Green Hungarian grapes from Acampo .....	29,200	6,603,000	.....	29,200	.....
4. Zinfandel grapes picked near winery .....	59,000	28,000	.....	less than 100	.....
5. Alicante Bouschet from Acampo .....	1,300	958,000	2,000	less than 100	.....
<b>1912:</b>					
6. Mataro picked near winery .....	1,300,000	.....	650,000	a few	.....
7. Zinfandel picked near winery .....	2,200,000	.....	1,300,000	a few	.....
8. Burger from Ripon (3 days en route) .....	9,200,000	.....	4,700,000	500,000	800,000
9. Alicante Bouschet from Ripon (3 days en route) .....	2,500,000	.....	8,440,000	440,000	.....

\* Blank spaces in columns mean that no cells of the type listed in the respective column developed on plates of dilutions used. This does not indicate that no cells of this type were present.

These tests justify the statement that grapes ordinarily arrive at the winery with a preponderance of the undesirable organisms as compared with the desirable true wine yeast, *S. ellipsoideus*. The actual numbers of all molds, wild yeasts, etc., are less on grapes gathered near the winery than on those shipped considerable distances before crushing; but the ratio of molds and wild yeasts to true yeasts is greater on clean grapes than on moldy ones judging from the above tests. Therefore, there is just as much, or even a greater need for the use of pure yeast with clean grapes than with grapes in poor condition, although it is extremely desirable to check the growth of the undesirable and promote the development of the desirable types in both cases.

## VIII. EXPERIMENTS UPON THE CONTROL OF MICRO-ORGANISMS ON GRAPES FOR WINE MAKING

### BEFORE SHIPMENT

Burger and Alicante Bouschet grapes from the vineyard of J. Swett & Son were used in the tests discussed below.

Several boxes of Burgers were crushed into a barrel that had been sterilized with strong potassium metabisulfite solution and rinsed out with clean water to remove the metabisulfite. To the crushed grapes was added potassium metabisulfite at the rate of 12 ounces<sup>s</sup> per ton of grapes. This was added in the form of a 10 per cent solution. Four clean Mason fruit jars were filled with the crushed grapes and sulfited at the rates of 6, 8, 9, and 14 ounces per ton. The metabisulfite is decomposed by the tartaric acid of the grapes to give sulfur dioxide and cream of tartar.

Several bunches of Alicante Bouschet grapes were crushed into a Mason jar and were treated at the rate of about eight ounces of metabisulfite per ton. The treated Burgers and Alicante Bouschet were shipped from Ripon to Martinez in the same car with untreated grapes. Counts of the micro-organisms on the grapes before treatment and on the treated and untreated grapes at the winery upon their arrival three days later were made.

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<sup>s</sup> Twelve ounces of  $K_2S_2O_5$  (potassium metabisulfite) per ton corresponds to approximately 190 milligrams  $SO_2$  (sulfur dioxide) per kilogram of grapes.

TABLE 21  
EFFECT OF THE USE OF MODERATE AMOUNTS OF SULFUROUS ACID IN SHIPPING  
GRAPES FOR WINE MAKING

	Grapes	Mold cells per c.c. of must	Wild yeast cells per c.c. of must	True wine yeast cells per c.c. of must	Vinegar bacteria cells per c.c. of must
1. Burgers before treatment	.....	18,000	2,600	a few	none
2. Untreated Burger on arrival at cellar	.....	9,200,000	4,700,000	500,000	800,000
3. Sulfited Burger in barrel on arrival at cellar	.....	less than 1	less than 1	125,000	none
4. Alicante Bouschet before treatment	.....	320,000	17,500	2,500	none
5. Untreated Alicante Bouschet on arrival at cellar	.....	2,500,000	8,440,000	440,000	.....
6. Sulfited Alicante Bouschet on arrival at cellar	.....	200	30,000	40,000	none

The Burger grapes in the jars all arrived in good condition, *i.e.*, with no visible mold growth and no fermentation; in fact, none of the treated grapes were moldy or fermenting. The untreated grapes of both varieties shipped in the ordinary way were visibly moldy and in some bunches the odors of fermentation and acetic acid were noticeable.

The amounts of sulfurous acid used were small and very much below the legal amount of 350 milligrams per liter allowed in finished wine in France. Analyses of the samples for sulfur dioxide on arrival at the cellar are tabulated in table 24. The grapes in the barrel were sulfited twelve ounces per ton, which is the equivalent to 190 milligrams of sulfurous acid per kilogram. On arrival at the cellar they contained only 110 milligrams of total sulfurous acid per liter. In other words almost one-half of the sulfurous acid had disappeared during shipment.

TABLE 22  
SULFUROUS ACID IN TREATED GRAPES ON ARRIVAL AT THE CELLAR

Sample	Total sulfurous acid in milligrams per liter	Free sulfurous acid in milligrams per liter
1. Barrel of sulfited Burgers .....	110	45
2. Jar number 1, Burger.....	48	16
3. Jar number 2, Burger .....	60	20
4. Jar number 3, Burger .....	68	32
5. Jar number 4, Burger .....	160	64

It was thought possible that the sprinkling of a little dilute potassium metabisulfite solution over the grapes in the boxes before shipment might protect them against mold growth, etc. Accordingly, about 150 c.c. of a 5 per cent solution of potassium metabisulfite was sprinkled throughout the bottom, center, and top of each of several boxes of Burger grapes and these were shipped with the untreated grapes. Contrary to expectations, they arrived in about as poor condition as the untreated grapes. The cause for the ineffectiveness of the treatment probably was that the metabisulfite solution dried out rapidly during shipment and thus became of little use in checking mold growth.

*Discussion of Tables 19, 20, and 21.*—From the results of these tables it can be seen that the wild yeasts and molds increase enormously during shipment under the conditions of the above experiments, which represent a fair average of the present commercial methods of shipping. A moderate amount of sulfurous acid held the undesirable organisms in check so well that none of these could be found in one of the samples of treated grapes and only a few in the



other. The true wine yeasts were not entirely suppressed but actually increased in numbers, though fermentation had not started.

This method could be applied commercially by crushing the grapes into wooden or iron tank cars at the vineyard and at the same time adding sulfurous acid in the form of 6% sulfurous acid solution or potassium metabisulfite. For normal conditions and where it is desired to prevent all fermentation and growth of mold, etc., during shipping, it would be advisable to use from twelve to sixteen ounces of the metabisulfite or three-quarters to one gallon of a 6% sulfurous acid per ton of crushed grapes. If it should be desired to prevent only the growth of molds and wild yeasts, twelve ounces of the metabisulfite or three-quarters of a gallon of the sulfurous acid 6% solution per ton would be sufficient in all cases. If the grapes should undergo wine yeast fermentation no great harm would be done. In fact, one step further could be made and pure yeast as well as metabisulfite could be added, in this way making use of the cars as fermenters and insuring good fermentations. It is possible that too much tannin would be extracted in the shipment of white grapes by the crushing and sulfiting method, but if no fermentation should take place it is not likely that this would happen.

#### AFTER ARRIVAL AT CELLAR

From the tests recorded in Part VII, it may be seen that the grapes as ordinarily received at the cellar have on their surfaces large numbers of fermentation organisms, most of which are of no value in wine making, or are of no direct harm to the production of good wine. As the season progresses, the numbers of the true wine yeast may increase in dirty conveyors, in the boxes, etc.; but this is an uncertain method because the kind and the numbers of the yeasts present are not under control. After heavy rains such as in 1912, the numbers of all yeasts on the grapes are diminished by being washed off. Such grapes may undergo all manner of mold and wild yeast fermentations because of the lack of true wine yeast. This was actually the case in wineries in Sonoma County, California, in 1912.

For these reasons, it becomes advisable to apply methods of wine making that discourage the growth of the undesirable types and favor the development of the true wine yeast. Experiments carried out in 1911, 1913, and 1914 and fully discussed in Bulletin 230 and Circular 140 of the California Agricultural Experiment Station demonstrated that the use of moderate amounts of sulfurous acid and the subsequent application of selected pure yeast to the crushed grapes or defecated must gave uniformly good fermentations and sound wines.

## SUMMARY OF PARTS II TO VIII

1. Nineteen different organisms from California grapes have been studied and their properties described. The majority of these were found to be types that are harmful in wine making. The true wine yeasts were not of the best varieties. If the yeasts so far studied are to be taken as representative of California yeasts in general, wine makers should not rely on natural fermentations for the production of their wines.

2. Grapes were gathered in vineyards at Davis, El Centro, Fresno, Ripon, and Martinez under conditions excluding, as far as could be, the possibility of outside contamination. The numbers and character of the micro-organisms present in a unit volume of the crushed grapes from each locality were determined. In all cases the undesirable organisms enormously outnumbered the true wine yeasts. Grapes from Tulare in 1906, Davis in 1912, and El Centro in 1912 contained no true wine yeasts, indicating that such yeasts are very rare in regions where wine making is not being carried on.

3. On the surfaces of the green grapes examined were found mostly molds with very few or no yeasts of any kind. As the grapes ripened wild yeasts made their appearance. The true wine yeasts were the last to appear.

4. As the grapes stand in boxes after picking in the vineyard, or on cars during shipment to the winery, the organisms on their surfaces greatly increase. During this increase in numbers of cells the molds and wild yeasts still remain greatly in excess of the true wine yeasts.

5. Grapes as received at two wineries in 1911 and 1912 under normal working conditions contained large numbers of molds and wild yeast cells and in one case vinegar bacteria, but there were very few wine yeasts (*S. ellipsoideus*) in all cases, compared to the numbers of other organisms present.

6. The molds, wild yeasts, and bacteria may be eliminated from the grapes during shipment by crushing the grapes at the vineyard and adding moderate amounts of sulfurous acid. The amounts of sulfurous acid necessary are far below the limits allowed by law in wine making countries and a great deal of this disappears during shipment and fermentation.

7. After the grapes have arrived at the cellar, the development of the undesirable types of fermentation organisms may be checked by the use of sulfurous acid and good fermentations assured by the use of pure selected yeast.

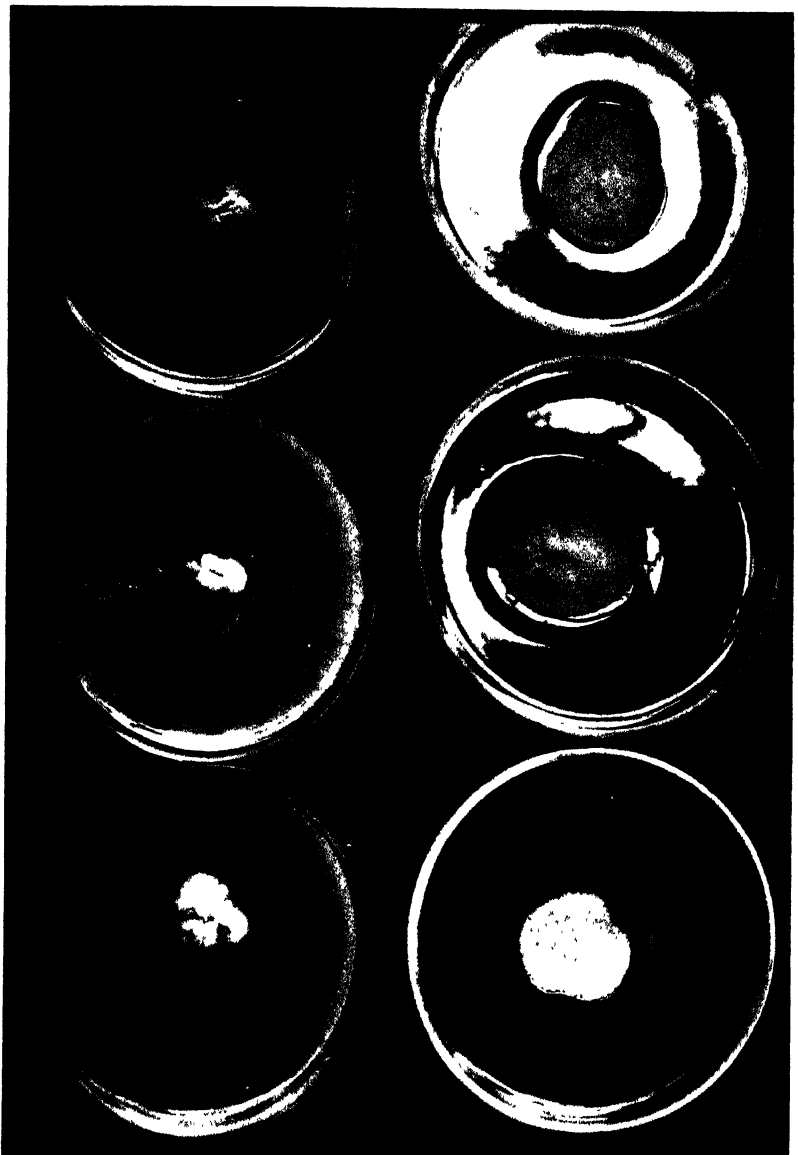
# PLATE 1

Apparatus used in isolating micro-organisms from grapes: Mason jar plugged with cotton and sterilized. Sterile petri dishes. Sterile 1 c.c. pipettes. Sterile agar and sterile must in tubes.



## PLATE 2

Colonies of yeasts 70, 71, and 78, on agar agar and gelatin. Upper row of plates agar agar; lower, gelatin. Reading from left to right colonies represent numbers 70, 71, and 78.



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TESTS OF CHEMICAL MEANS FOR THE  
CONTROL OF WEEDS

REPORT OF PROGRESS

BY  
GEORGE P. GRAY

UNIVERSITY OF CALIFORNIA PRESS  
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INTRODUCTION

The cultivation of crops has been actually abandoned on hundreds of acres of some of the most fertile land of the state and the productivity of thousands of acres more is rapidly decreasing through the increase and spread of wild morning-glory and Johnson grass. These weeds have defied practically all control measures so that their appropriation of still more choice land remains unchecked. The mechanical control methods commonly used against these two weeds are hand-digging and clean culture for at least a year. The expense of either of these procedures is always great and sometimes there must be added the loss of the use of the land during the process. The expense involved may equal or exceed the value of the land, but the most serious aspect of the matter is that too often the efficiency of the hand-digging or clean culture operations may fall below a full hundred per cent, in which case the attempt usually results in a complete failure. Any measure short of complete eradication does not, as a rule, justify the expense.

The two plants mentioned are undoubtedly foremost among the weed pests of the state, but all weeds exact a heavy tribute from the tiller of the soil. Other noxious weeds of great economic importance are: the so-called "water-grass" of rice fields, Bermuda grass, wild mustard, wild radish, foxtail, and thistles. These and others have been found very difficult to control and under some conditions their increase and spread has been so rapid as to make the cultivation of the infested fields unprofitable.

Certain of our statutes declare noxious weeds to be a nuisance and empower the county horticultural commissioners to enforce the abatement of such nuisances. The enforcement of these laws, however, is very difficult and in certain sections has not been attempted on account of the magnitude of the undertaking, the expense involved, or the uncertainty of success of the known means commonly used toward eradication.

These conditions constitute a very serious menace to the agriculture of the state and were fully discussed at a meeting of the county horticultural commissioners of California held at Stanford University, July 26, 1915. The use of chemicals was frequently mentioned as a possible solution of the weed problem, but the fact was brought out that very little information is available concerning their usefulness under California conditions. Following this meeting, Mr. William Wood, Los Angeles County Horticultural Commissioner, took up with the director of the agricultural experiment station at Berkeley the matter of an investigation of methods of weed control by means of chemicals. It was suggested that a judicious use of chemicals might solve the problem of the control of those weeds which too often survive and increase in spite of expensive mechanical control measures. The suggested investigation was undertaken by the station in the fall of 1915, and the writer was assigned to the work.

A review of the literature discloses the fact that the use of chemicals has simplified to some extent the solution of the problem of weed control on both agricultural and non-agricultural land in localities other than California.\* In one case at least, the use of chemicals for the destruction of weeds has become a common practice.<sup>9</sup> Some of the experiments have demonstrated the usefulness of chemicals for the destruction of weeds on a large scale. Methods which have proved efficient elsewhere may also prove to be efficient here. The soil and climatic conditions of California, however, are so different from those of regions where the chemical method has proved satisfactory, that it is by no means certain that this will be the case. A study of the literature gave encouragement that weed pests might be controlled under local conditions if the conditions were carefully studied and the treatment made accordingly.

Herbicide experiments have been made by several of the railroad companies operating in the state. Probably the first of the county horticultural commissioners to experiment with chemicals for the

\* See references on page 97.

control of weeds was Mr. F. W. Waite of El Centro. Other commissioners who have tried this method are Mr. William Wood of Los Angeles, Mr. A. A. Brock of Ventura, and Mr. C. W. Beers of Santa Barbara. Very few data have been published upon the results of these experiments, but the writer has secured much valuable information and confirmatory evidence through correspondence with these experimenters and by occasional inspection of their results. In general, the results were encouraging, but sufficient progress had not been made to establish a definite mode of procedure.

#### INVESTIGATIONS BY THE INSECTICIDE AND FUNGICIDE LABORATORY

The following progress report describes the more important experiments which have been made during the investigation. A mass of data has been accumulated showing the effect of a number of substances on plant life and on soils, some of which have been sufficiently conclusive to warrant the recommendation of control measures. Some of the experiments have opened up a very promising field for further investigation, while others have been largely negative in character. No attempt is made in this account of the experiments to make definite recommendations for control measures. The practical application of the results is reserved for discussion in separate publications on special topics.\*

#### ACKNOWLEDGMENTS

An account of the investigations would be incomplete without special mention of the work of two student assistants, Mr. C. C. Barnum and Mr. T. M. Pierce. Their assistance in the planning and execution of the experiments, and in the interpretation of the data, has in no small way contributed to the preparation of this report. Mr. M. R. Miller and Mr. W. C. Matthews have added much of value in photographic records. A number of the county horticultural commissioners, their deputies and inspectors, have freely given advice and both their time and transportation facilities whenever needed. Mr. Fred Lowrie and Mr. George Lowrie have generously allowed the use of land and a pumping plant for experimental purposes.

\* Circular 168 of this station, "Spraying for the control of the wild morning-glory within the fog belt," is now available for distribution and may be had upon application to the director.

Mr. F. E. Sullivan, Mr. Geo. T. Scott, and other officials of the Spreckels Sugar Company have shown a hearty spirit of coöperation and have made possible the accumulation of many valuable data by Mr. A. M. Hunt and Mr. H. K. Fox, to whom was assigned the execution of the detail of the coöperative experiments in the Salinas Valley.

#### SCOPE OF INVESTIGATIONS

The wild morning-glory is undoubtedly the most difficult to control of any of the weed pests of the state. It is widely distributed and is rapidly spreading. The known methods of control are probably the least satisfactory of those for any weed. The greatest need for investigation appeared to be the control of this pest. The principal part of the problem attacked, therefore, was the control of wild morning-glory upon agricultural land without injury to the soil.

#### METHODS OF USING HERBICIDES

Chemicals have been used for the control of weeds in at least two ways in which the manner of action of the poison on the plant is fundamentally different. A well defined conception of this difference in principle is essential to the formulation of control measures. One or the other may be the more suitable procedure to adopt, depending on various conditions. The experiments hereafter reported include tests of these two methods, which, for the purpose of discussion will be referred to as: the "root-absorption method," and the "leaf-absorption method."

#### TESTS OF HERBICIDES BY ROOT-ABSORPTION METHOD

Arsenic, sodium cyanide, sulfuric acid, acid sludge, acid tar, salt, carbon bisulfide, copper sulfate, and iron sulfate were tested against wild morning-glory by the root-absorption method. These were applied so that the soil in the vicinity of the roots of the weeds was more or less permeated with the herbicide. In this method the poisons used per square yard varied from one-half an ounce to three and one-half pounds in testing some materials. Experimental plots were located at Whittier, Centerville, and at Spreckels.

## AT WHITTIER AND CENTERVILLE

The first experiments were made at Whittier, September 10, 1915, but conclusions could not be drawn on account of the short duration of the observations. They did indicate, however, that sodium arsenite and sodium cyanide were worthy of a further trial against morning-glory and Johnson grass, and that salt applied at the rate of  $3\frac{1}{2}$  pounds per square yard, under the conditions of the experiment, was of no value against the above weeds.

Experiments were made later on the Lowrie Ranch at Centerville, designed principally to test the effect of the root absorption of four materials on morning-glory and the effect of these materials on the soil.

*Experimental Plots.*—The land assigned for the experimental plots was level, and uniformly and heavily infested with wild morning-glory, the vines forming a dense mat, in most places, four or five inches thick. The soil was visible in a few spots only. No other vegetation was growing on the land at the time of the treatment. A crop of garlic had been grown previously on the land and harvested in May or June, 1915.

The land was marked off into rows 1 yard wide and 10 yards long, each row being divided into ten plots of a square yard each. The rows were lettered A, B, C, etc., and the plots of each row numbered 1 to 10, inclusive.

*Chemicals Tested.*—On October 9, 1915, different treatments were made on 93 plots. The four materials tested were:

1. Arsenic in the form of sodium arsenite: a stock solution was prepared by dissolving arsenic trioxide in sodium hydroxide and water in such proportions that each gallon contained the equivalent of 4 pounds of arsenic trioxide.\*

2. Sodium cyanide: a stock solution was prepared by dissolving the solid in water and diluting so that each gallon contained 2 pounds of sodium cyanide.

3. Sulfuric acid (commercial 66° Baume): a powerful corrosive to vegetable tissue.

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\* The stock solution formula is:

White arsenic (arsenic trioxide 99%) .....	20 pounds
Granulated caustic soda (98%) .....	10 pounds
Water, to make .....	5 gallons

Detailed directions for the preparation of the above stock solution are given in Circular 168 of this station, Spraying for the control of wild morning-glory within the fog belt.

4. Acid sludge: until recently a waste product in the refining of petroleum distillates with sulfuric acid. Previous unpublished experiments of Mr. E. R. de Ong and the writer to study the action of petroleum oils on citrus and other foliage had indicated that the constituents of petroleum distillates which are capable of being removed by refining with sulfuric acid are much more toxic to foliage than other constituents. It was thought that acid sludge, therefore, containing these highly toxic constituents of petroleum as well as an excess of sulfuric acid, might prove to be an efficient and economical herbicide.

*Manner of Application.*—For each treatment a quantity of the stock solution or liquid was measured out and diluted to a volume of either one or two gallons. All of the diluted solution was then uniformly distributed over one of the square-yard plots by means of an ordinary garden sprinkling pot. It was found by preliminary trial that a gallon of water applied to a square-yard area of the soil would thoroughly soak the ground for about an inch below the surface. This quantity was therefore applied to some of the plots; on others the amount of liquid was doubled in order to ascertain whether or not the greater amount of liquid would result in permeating the soil with the poisons to a greater depth than the smaller amounts. It was thought that the vines might affect the absorption of the poisons, so the vines were removed from some of the plots before applying the solutions.

Table 1 illustrates in detail the treatment given the individual plots.

#### AT SPRECKELS

• Coöperative experiments with the Spreckels Sugar Company were started in March, 1916, on its ranches in the Salinas Valley.

The materials tested on morning-glory were: iron sulfate, copper sulfate, acid sludge,\* acid tar\* (derived from acid sludge), sulfuric acid, carbon bisulfide, and "Nonpariel" (a commercial herbicide, consisting principally of carbon bisulfide).

Application of the materials was made on square-yard plots in the same manner as in the Centerville experiments with the exception of the carbon bisulfide and Nonpariel. These were applied by making holes on the plots at intervals with an iron rod and pouring into the holes a quantity of the material and immediately filling the holes with earth.

\*Furnished by courtesy of the Union Oil Company of California.

TABLE 1  
TREATMENTS ON 93 SQUARE-YARD PLOTS AT CENTERTVILLE, OCTOBER 9, 1915.

The large figures indicate the amount in ounces of the material applied to each plot, solids expressed in ounces avoirdupois, and liquids in fluid ounces

Material applied	Row	Plot numbers										Treatment of row
		1	2	3	4	5	6	7	8	9	10	
Arsenic trioxide .....	A	16	12	8	7	6	5	4	3	2	1	2 gallons solution; vines on.
Arsenic trioxide .....	B	16	12	8	7	6	5	4	3	2	1	2 gallons solution; vines removed.
Arsenic trioxide .....	C	8	6	4	3½	3	2½	2	1½	1	½	1 gallon solution; vines on.
Arsenic trioxide .....	D	8	6	4	3½	3	2½	2	1½	1	½	1 gallon solution; vines removed.
E												
Check												
Sodium cyanide .....	F	16	12	8	7	6	5	4	3	2	1	2 gallons solution; vines on.
Sodium cyanide .....	G	16	12	8	7	6	5	4	3	2	1	2 gallons solution; vines removed.
Sodium cyanide .....	H	8	6	4	3½	3	2½	2	1½	1	½	1 gallon solution; vines on.
Sodium cyanide .....	I	8	6	4	3½	3	2½	2	1½	1	½	1 gallon solution; vines removed.
J												
Check												
Sulfuric acid .....	K	24	20	16	12	8	4	....	....	....	....	1 gallon solution; vines on.
Acid sludge .....	L	20	16	12	8	4	2	....	....	8*	....	1 gallon solution; vines on.

\* Two gallons of solution applied

## CONCLUSIONS CONCERNING ROOT-ABSORPTION METHOD

Inasmuch as the root-absorption method gave little promise of offering a solution of the morning-glory problem, space will not be taken for a detailed report of the results. Table 2 will be of interest in showing the cost of the more effective materials tested. Costs shown in this table and elsewhere in this report are calculated from the retail prices prevailing at the time of the treatments.\* The cost of solids (arsenic trioxide and sodium cyanide) is given in ounces avoirdupois; the cost of liquids (sulfuric acid and carbon bisulfide) is given in fluid ounces, using the figure 1.8 as the specific gravity of commercial sulfuric acid, and 1.27 as that of carbon bisulfide.

TABLE 2

COST OF MORE EFFECTIVE MATERIALS TESTED BY ROOT-ABSORPTION METHOD

	Arsenic trioxide	Sodium cyanide	Sulfuric acid	Carbon bisulfide
Cost per ounce as applied, approximate ...	\$0.008	\$0.020	\$0.0025	\$0.006
Least amount per square yard giving any promise of control of morning- glory, ounces .....	3	3	20	10
Cost per square yard .....	\$0.024	\$0.06	\$0.05	\$0.06
Cost per acre .....	117.90	290.40	242.00	305.50
Least amount per square yard prevent- ing the growth of practically all vege- tation except morning glory for 14 months on undisturbed soil, ounces ..	1	12	24	Soil not injured by this material
Cost per square yard .....	\$0.008	\$0.24	\$0.06	.....
Cost per acre .....	39.30	1,160.60	290.40	.....

Cost of the materials in table 2 are calculated from the following prices: Arsenic trioxide, @ 8c per lb.; sodium cyanide, @ 32c per lb.; carbon bisulfide, @ 90c per gal.; caustic soda, @ 10c per lb.; sulfuric acid, @ 2c per lb.

No data are available on the cost of acid sludge and acid tar. Until their recent use in the flotation process for the concentration of certain low grade ores, they were both waste products in refining petroleum distillates and had very little commercial value.

Nonpareil sells at about the same rate as carbon bisulfide or cheaper.

No sweeping conclusions are justified from the results of the experiments. Whether or not the results would be duplicated on other soils, or under different climatic conditions, or if the chemical were applied at other seasons of the year as yet can not be predicated.

\* These prices are above normal. Since the preparation of the manuscript, the price of arsenic has advanced to 23 cents per pound. Other materials are also higher and some are difficult to obtain at any price. The present unsettled condition of the market does not warrant a revision of prices.



## CONTROL OF WILD MORNING-GLORY

None of the root-absorption experiments seemed to point the way for the control of wild morning-glory on agricultural land at a reasonable expense and without serious injury to the soil. In the experiments, and in all cases that have come to the attention of the writer, where a chemical has been used by this method, the cost would prohibit its use as a *control* measure, except on small areas; if *eradication* has not been accomplished, the results have not justified the means. Furthermore, it was found that in using a non-volatile herbicide (such as salt, arsenic, sodium cyanide, sulfuric acid, etc.), if the soil is sufficiently

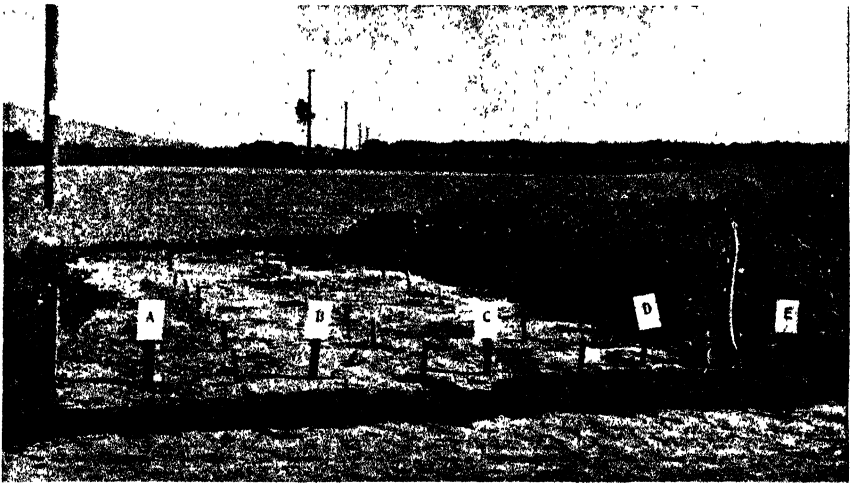


Fig. 1.—The effect of arsenic on the soil when applied by the root-absorption method (see table 1).

“Incidental to the main object of the experiments, . . . data . . . show the superiority of arsenic as a soil sterilizer.”

permeated with the chemical to destroy the roots of the wild morning-glory, it is rendered unfit for the growing of crops for many months.\* Carbon bisulfide, on the other hand, is a volatile herbicide and appears to produce no injurious effects on the soil. It is also quite effective against morning-glory, but the expense of application and the cost of material are both high.

## SOIL STERILIZATION

Incidental to the main object of the experiments, the control of wild morning-glory on agricultural land, data have been obtained which show the superiority of arsenic as a soil sterilizer (compare figures 1, 2, and 3). While arsenic has apparently failed to actually eradicate

\* See further discussions of the effects of arsenic on the soil on pages 90-91.

the wild morning-glory, using as high as a pound to the square yard, it can be used as a soil sterilizer in respect to a great variety of weeds. All of the Centerville plots to which an ounce or more of arsenic trioxide had been applied per square yard were barren of all vegetation, except morning-glory, for fourteen months (fig. 1), notwithstanding the leaching by the rains of two winters. The minimum cost of materials for the production of this result was less than one cent per square yard or about forty dollars per acre.\* This would not be prohibitive for the prevention of weed growth on gravelled walks, tennis courts,



Fig. 2.—The effect of sodium cyanide on the soil when applied by the root-absorption method (see table 1).

On all plots receiving six ounces or less of sodium cyanide the final growth of miscellaneous weeds was fully equal to that on the checks. The smaller amounts appeared to stimulate the growth.

roadways, fencerows, or in other places where soil sterilization is desired.

One significant fact in connection with soil sterilization is that any amount of arsenic in excess of one ounce per square yard appears to have been a waste of material unless future observations will show that larger amounts will have a more lasting effect.

The experiments have furnished a basis for further experiments in the problem of weed control on railroad right-of-ways. This matter has already received considerable attention by a number of railroads, but the danger of poisoning live stock has had a restraining influence

\* See footnote on page 74.

on the use of arsenic for weed control in this connection. The suggestion to add some substance to the herbicide as a repellant to stock has been given attention, but sufficient progress has not yet been made to warrant publication.

## TESTS OF HERBICIDES BY LEAF-ABSORPTION METHOD

The preceding experiments are in sharp contrast to those reported below. In the former, an attempt was made to poison the soil in the



Fig. 3.—The effect of sulfuric acid on the soil when applied by the root-absorption method (see table 1).

vicinity of the root system; in the latter, the poisons were not applied to the soil, but were sprayed on the foliage of the weeds. For the purpose of discussion, the latter method will be referred to as the "leaf-absorption method." The amount of poison required in this method is very small so that even though a very poisonous substance is used, the quantity will be so evenly distributed over the area treated that the probability of injury to the soil is reduced to the minimum. It has been demonstrated that wild mustard, wild radish, and other annual weeds can be economically controlled in the grain fields of the middle west by spraying with a solution of iron sulfate or of copper sulfate.<sup>1, 2</sup> A dilute spray of sodium arsenite is extensively employed in the Hawaiian Islands for the control of weeds on sugar, rubber, and

pineapple plantations.<sup>4, 9, 10</sup> Experiments have shown that certain oil sprays are effective for the control of wild onion and wild-garlic in Indiana and Ohio.<sup>7, 11</sup> Experiments in Australia have proved the effectiveness of an arsenical spray for the destruction of cacti.<sup>5</sup> In the literature just cited, the leaf-absorption method has been demonstrated to be effective for the control of weeds on a large scale. So far as known, however, this method has not been tried against the wild morning-glory, a persistent and deep-rooted perennial (figs. 4 and 5). Bioletti<sup>8</sup> reports finding roots of this weed well supplied with starch



Fig. 4.—A remarkably heavy infestation of wild morning-glory covering about two acres in the center of a beet field.

The stand of vines was well above one's knees.

and capable of producing new shoots at a depth of fourteen feet, the smallest piece of which is capable of growing and originating a new plant.

#### PRELIMINARY EXPERIMENT AT CENTERVILLE

Following the tests of the root-absorption method at Centerville, a test was made at the same place of the leaf-absorption method so much in use in Hawaii.<sup>9</sup>

*Experimental Plot.*—The plot selected for the experiment was immediately adjoining the plots used for the root-absorption experiments, and consisted of 51½ square yards, quite uniformly and heavily infested with morning-glory, the vines forming a dense mat four or five inches thick in most places. No other vegetation was growing on the plot at the time of treatment. A crop of garlic had been grown on the land and harvested in May or June, 1915. The plot was designated as Spray Plot 1.

*Chemical Tested.*—A dilute arsenical solution was prepared by mixing 4 fluid ounces of the arsenic stock solution (p. 71) with 3 gallons of water. This made a solution roughly equivalent to a dilution of the stock solution 1:100, or 4 pounds of arsenic trioxide per 100 gallons. The three gallons contained the equivalent of 2 ounces of arsenic trioxide.

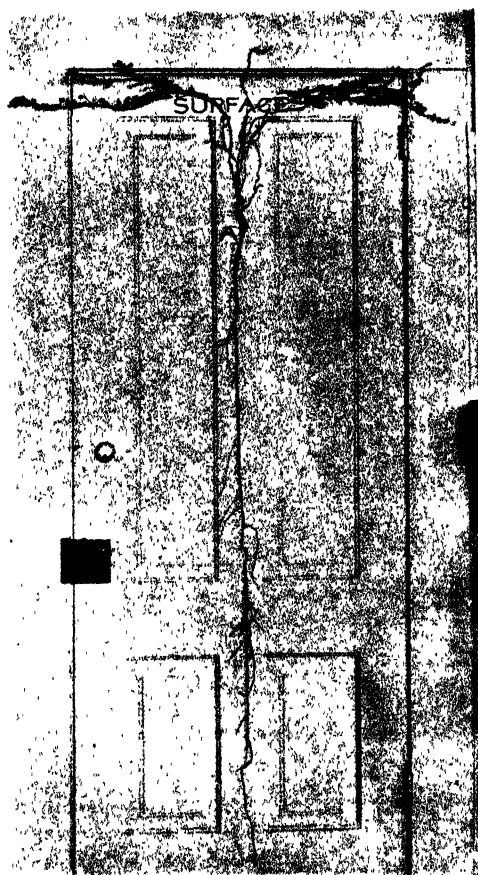


Fig. 5.—Vines and root of wild morning glory.

*Manner of Application.*—The solution was applied November 5, 1915, to the morning-glory vines on the plot by means of a bucket spray pump. Just enough of the spray was used to moisten the leaves and stems of the vines only, no attention being given to the few bare spots where the soil was visible. The 3 gallons of solution was found sufficient for the purpose, the whole plot of  $51\frac{1}{2}$  square yards thus receiving only 2 ounces of arsenic trioxide.

*Results.*—One week after the application of the spray the morning-glory vines were found completely killed to the ground, but the roots appeared to be normal. A similar observation was made a week later. Three weeks after spraying, however, the effects observed on the roots of the morning-glory vines were remarkable. The usual new growth of sprouts from the roots after the destruction of the vines had not occurred. The roots were moldy and disintegrating to a depth of several feet below the surface. Forty-five days after spraying, only nine plants had produced any new growth above ground on the whole plot, while it was estimated that fully six hundred plants were above the surface on an equal area of the adjoining field which had been recently gone over with the weed knife. The usual winter growth of wild grasses and other weeds was well started in the vicinity, the growth of which on the sprayed plot was fully equal to that on the adjoining plot.

*Comments.*—This experiment indicated that the leaf-absorption method for the control of morning-glory on agricultural land has great possibilities. The cost of materials did not exceed \$1.50 per acre,\* the poison having been applied at the rate of 12 pounds per acre; the remedy was applied as a spray, which is probably the most economical manner of application; the apparent control of the weed at a comparatively trifling expense was almost equal to the best results obtained in any of the root-absorption method experiments; and no ill effects to the soil were observed. The possible utility of this method was also noted for the control of miscellaneous weeds upon non-agricultural land where cultivation is difficult or impossible.

In view of the results obtained in the first test of the leaf-absorption experiment it seemed desirable to make as thorough a study as possible of this method and to secure sufficient land for the purpose with reasonable assurance that the experiments would not be molested.

#### PRELIMINARY EXPERIMENTS AT DAVIS

A series of experiments at the University Farm at Davis was outlined and work started before the occurrence of the winter frosts of 1915.

*Experimental Plots.*—Most of the land assigned for the experiments was well infested with morning-glory. It had been used in the summer of 1915 for a crop of vegetables. No plants other than morning-glory were growing on the plots when the experiments were begun.

The land to be used for our tests was divided into thirty plots

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\* See footnote on page 74.

of one-fortieth of an acre each. The experiments were outlined, principally, to determine the most suitable of three forms of soluble arsenic and the time of the year to obtain the best absorption of these materials through the aerial parts of the weeds.

*Chemicals Tested and Manner of Application.*—On December 23, 1915, one of the plots was treated in exactly the same manner as described in the preliminary experiments at Centerville. A dilute arsenical solution was prepared by mixing  $9\frac{1}{3}$  fluid ounces of the arsenic stock solution described on page 71 with 7 gallons of water. Another plot was sprayed the same day with a solution of the same concentration of arsenic but made up with half the quantity of caustic soda. A third was sprayed the following day with a solution of *arsenic acid* containing the same amount of actual arsenic as the two previously described sprays.

*Results Complicated the Problem.*—Repeated observations subsequent to the application of these sprays at Davis failed to disclose any injury to the roots of the morning-glory vines on any of the plots. The striking results of the Centerville experiment and the failure of similar treatments two months later at Davis clearly suggested the importance of making a study of all conditions which might affect the results. It was thought that the climate, the season of the year, the weather at or near the time of application, the condition of the plants, the type and condition of the soil, individually or collectively, and possibly others, might be factors influencing the results to a greater or less extent.

#### PLAN OF EXPERIMENTS IN 1916

The problem at once became so complicated that it seemed wise to limit the future experiments to a study of the leaf-absorption method, and to pay most attention to making a thorough test of the arsenical spray treatment under as many conditions as possible. The winter rains and the killing of the morning-glory vines by the frost prevented any further experiments until March, 1916, at which time new ones were started.

Comprehensive series of experiments were carried on during the growing season of 1916 and until the vines were killed by the frosts. The killing frosts of 1916 occurred earlier than those of 1915 so that the experiments had to be discontinued at an earlier date. The Centerville and Davis experiments were continued and coöperative experiments in the Salinas Valley in coöperation with the Spreckels Sugar

Company were also started, as well as a series of experiments at Berkeley.

Experimental plots were thus located on different types of soil in different localities, upon soil of the same type but differing in drainage conditions, and upon both lightly and heavily infested plots. The weather conditions at the various experimental plots were different. The climate at Davis is semiarid, while the remainder of the plots were within the fog belt, although differing somewhat in distance from the coast. The details of all the experiments of 1916 will not be described in full in this progress report but only such observations as seem to be the most significant.

#### FURTHER EXPERIMENTS AT CENTERVILLE

The experiments at Centerville were continued on more infested land adjoining the plots previously used.

*Outline of Experiments and Subdivision of Plots.*—In order to make observations on the results to be obtained by repeated sprayings at different intervals, Spray Plot 1 was divided into two parts, A and B. Spray Plot 1A was further divided into five small plots (1 by 5 yards) which were lettered a, b, c, d, and e; certain ones to be resprayed at irregular intervals, depending upon the growth of weeds.

Spray Plot 1B to be resprayed once in the fall of 1916.

Convenient sized plots (2 by 10 yards) were staked out adjoining Spray Plot 1, to be sprayed at different times of the year.

The manner of subdivision and the dates on which the sprays were applied are indicated in tables 3 and 4.



TABLE 3  
SPRAY PLOTS 1A AND 1B, SHOWING DATES OF SPRAYING

<div><div>Sprayed</div><div>11/5/15</div><div>10/14/16</div></div> <div><div>Sprayed</div><div>10/14/16</div></div> <div><div>←</div><div>B</div><div>→</div></div>				
<div>a</div> <div>Sprayed</div> <div>11/5/15</div> <div>3/11/16</div> <div>5/5/16</div> <div>6/16/16</div> <div>7/15/16</div> <div>8/15/16</div> <div>10/14/16</div>	<div>b</div> <div>Sprayed</div> <div>11/5/15</div> <div>3/11/16</div> <div>5/5/16</div> <div>6/16/16</div> <div>7/15/16</div> <div>10/14/16</div>	<div>c</div> <div>Sprayed</div> <div>11/5/15</div> <div>3/11/16</div> <div>5/5/16</div> <div>10/14/16</div>	<div>d</div> <div>Sprayed</div> <div>11/5/15</div> <div>3/11/16</div> <div>10/14/16</div>	<div>e</div> <div>Sprayed</div> <div>11/5/15</div> <div>10/14/16</div>
<div><div>←</div><div>A</div><div>→</div></div>				

TABLE 4

## SPRAY PLOTS 2 to 7

SOUTH OF AND ADJOINING SPRAY PLOT 1. PLOTS 2 BY 10 YARDS CHECKS  
1 BY 10 YARDS. SHOWING DATES OF SPRAYINGS

R	CHECK	Sprayed 10/14/16	
S	<b>Spray Plot 2</b>	Sprayed 3/11/16 and 10/14/16	
T			
U	<b>Spray Plot 3</b>	Sprayed 6/16/16 and 10/14/16	
V		.	Garlic field in 1915
W	CHECK	Sprayed 10/14/16	
X	<b>Spray Plot 4</b>	Sprayed 7/15/16 and 10/14/16	
Y			
Z	<b>Spray Plot 5</b>	Sprayed 8/15/16 and 10/14/16	
			Tomato field in 1916
	CHECK	Sprayed 10/14/16	
	<b>Spray Plot 6</b>	Sprayed 9/16/16 and 11/11/16	
	<b>Spray Plot 7</b>	Sprayed 10/14/16	
	CHECK	Sprayed 11/11/16	

*Chemicals Tested and Manner of Application.*—The sprays were all made up and applied in the manner described in the preliminary experiment at Centerville. Four fluid ounces of the arsenic stock solution (p. 71) were mixed with 3 gallons of water. The solutions were applied by means of a pressure sprayer, using just enough to moisten the foliage of the weeds. The quantity required for the purpose was

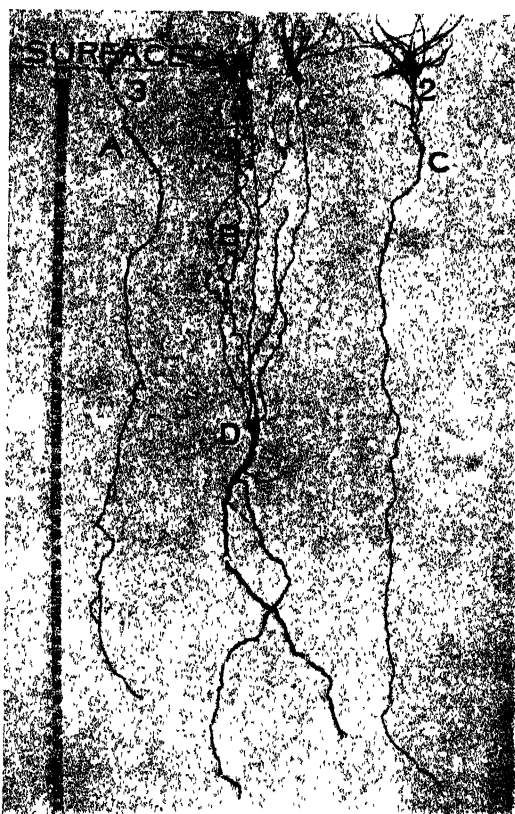


Fig. 6.—Typical root systems of morning-glory plants at Centerville, destroyed by an arsenical spray applied to the aerial parts only.

Spray applied September 16, 1916; roots dug, October 14, 1916. Root 1 was dissected out to a distance of 4 feet, 2 inches below the surface and was one-half inch in diameter at D.

roughly equivalent to 300 gallons per acre, containing sodium arsenite equivalent to 12 pounds of arsenic trioxide, and 6 pounds of sodium hydroxide. The cost of materials for the 300 gallons of spray was estimated to be \$1.50 at retail price prevailing at the time of treatment.\*

\* See footnote on page 74.

*Results.*—Each spraying entirely killed the vines of the morning-glory to the surface of the ground, as well as most of the broad-leaved plants. The grasses were much less affected. The roots of the morning-glory were not materially injured by any spray applied between March and August, 1916, but the spray applied August 15, 1916, caused marked injury to the roots of the morning-glory. The usual growth of new sprouts was markedly less than usual, only 18 having reached the surface within one month after spraying. Even these seemed to be lacking in vitality. Many roots were apparently dead from  $1\frac{1}{2}$  to 2 feet below the surface of the ground.

The effect of the spray applied September 16, 1916, was still more encouraging. One month after the application of the spray, a hole



Fig. 7.—Showing the injured condition of the interior of a section of the lower end of root 1, figure 6, four feet below the surface.

was dug on the plot to a depth of five feet. Working laterally from this hole, largely by means of trowel and geology pick, the soil was removed from some twenty-five roots (see fig. 6). Only one of these roots appeared to be normal. The rest were shrivelled, moldy, and disintegrated from the base of the vine stems to two or three feet below the surface, the remainder still being plump. On exposing the interior of these plump lower sections of the roots, the cambium was found to be turned to a chocolate brown color and a very pronounced "sour" odor was given off. Figure 7 will give an idea of the appearance of the interior of a section taken from the lower end of root 1, figure 6, four feet below the surface.

*October Sprayings Most Effective.*—All plots previously sprayed (with the exception of Spray Plot 6) were sprayed again October 14, 1916, and the check plots between were also sprayed in order to make observations on the efficiency of two sprayings at different intervals

and to make the attempt to disinfest all the experimental plots. The October spraying appeared to be more effective than any previous one.

On November 11, 1916, a count was made of the morning-glory plants producing new vines on the various plots and their condition was noted with the results shown in table 5.

TABLE 5  
OBSERVATIONS ON CENTERVILLE PLOTS

Spray plot No	When sprayed	Observations, Nov. 11, 1916 Number of vines	Condition of vines
1	Plot 1B Nov., 1915, Oct., 1916 .....	27	Sickly
	Plot 1A (see map, p. 83).		
Check	October, 1916 .....	0	
2	March and October, 1916 .....	8	Sickly
3	June and October, 1916 .....	10	Sickly
Check	October, 1916 .....	3	Sickly
4	July and October, 1916 .....	5	Sickly
5	August and October, 1916 .....	40	Vigorous
Check	October, 1916 .....	0	
6	September only, 1916 .....	34	Sickly
7	October only, 1916 .....	5	Sickly
TOTAL .....		132	
Total area included in the above count .....		200 sq.yds.	
Estimated number of plants on this area before treatment ...		2,400	
Total number of plants producing new vines on this area after treatment .....		132	
Numbered plots, 2 by 10 yards; checks, 1 by 10 yards.			

#### RÉSUMÉ OF LEAF-ABSORPTION EXPERIMENTS

The experiments at Davis were continued during 1916 until the vines were killed by the frost early in November. The three arsenical sprays (p. 81) were applied to additional plots in the months of March, May, June, July, August, September, and October. None of the sprayings produced very satisfactory results. A comparison of the efficiency of the three forms of arsenic is, therefore, impossible. In some of the experiments the arsenic acid spray seemed to be more severe in its action than others.

The Spreckles Sugar Company has provided for the full time of one man since March, 1916, for coöperative experiments on their ranches in the Salinas Valley. Arsenical sprays of different concentrations were applied to morning-glory every month of the year from March to December. The problem of the control of miscellaneous weeds

along their extensive irrigation system and along roadways was also attacked. Arsenic was given the most extensive trial, and, in addition, sodium cyanide, iron sulfate, copper sulfate, acid sludge, and acid tar were tested.

A number of experiments were also made at Berkeley, testing the arsenical spray on morning-glory.

Each set of experiments has contributed materially in throwing light on the problem of the control of weeds by means of chemicals. The results of the above experiments are summarized under the various headings below.

#### PETROLEUM PRODUCTS AS HERBICIDES

While arsenic was found to be not only the most potent of the plant poisons thus far tested, but also the cheapest, there are certain dangers attending its use which must not be overlooked. This fact has led the writer to make a search for an herbicide which is less poisonous to man and animals, and yet not prohibitive in price. The experiments in this direction were rather limited but were sufficiently encouraging to warrant further investigation.

A spray composed of equal parts of acid sludge (p. 72) and water, applied to wild morning-glory vines in October, 1916, appeared to be as effective as arsenic in destroying the weed. Acid tar (a derivative of acid sludge containing less free acid) gave encouraging results on morning-glory.

The above materials applied as a spray, either pure or diluted, were especially effective on grasses. A spray of acid tar applied to a plot of succulent grass (principally foxtail and wild oats) caused withering and browning of the leaves within half an hour after application; within twenty-four hours the grass readily took fire from a lighted match and burned.

Petroleum-distillate sprays were tested on miscellaneous weeds. The results indicated that certain of these distillates were more effective in destroying grasses than sodium arsenite, while sodium arsenite was generally more effective on the broad-leaved plants than the distillates. The cheaper grades of distillates were more effective than refined distillates such as kerosene and similar products. Petroleum products containing a large percentage of aromatic and "cracked" oils were more effective than those containing a lesser percentage.

## ARSENIC ON MISCELLANEOUS ANNUAL WEEDS

The growth of many annual weeds was easily killed to the ground by spraying with a dilute arsenical spray. The roots were not always destroyed and would often produce a new growth.

Annual weeds of more tender foliage were destroyed with a spray containing the equivalent of four pounds of arsenic trioxide per hundred gallons. The grasses were more resistant to arsenic than most other weeds. These and weeds of more hardy foliage seemed to require a more concentrated spray than the above. Sprays containing the equivalent of eight to twenty pounds of arsenic trioxide per hun-

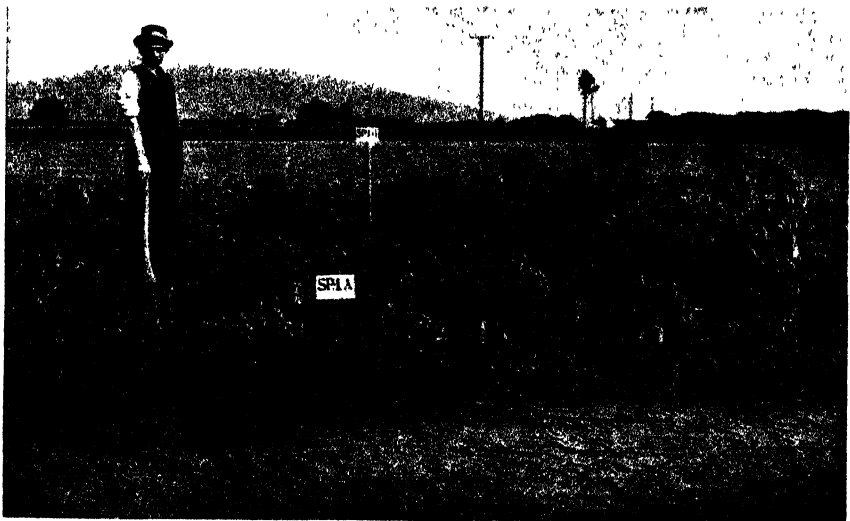


Fig. 8.—The effect of arsenic on the soil when applied by the leaf absorption method (see table 3).

Spray Plot 1A had been sprayed twice, November 5, 1915, and March 11, 1916; and Spray Plot 1B had been sprayed once, November 5, 1915, when the photo was taken, April 28, 1916. The growth of miscellaneous weeds on the plots after treatment has been used throughout the experiments as an indication of the effect of the treatments on the soil.

dred gallons have usually given satisfactory results in the control of the more resistant annual weeds. The optimum concentration was not determined, but it appears that it will have to be varied according to the nature of the weeds.

The arsenical-spray method has been shown to be suitable for the control of miscellaneous annual weeds growing along irrigation ditches, fencerows, and other places where cultivation is difficult or impossible and for the prevention of the maturing of seeds of perennial noxious weeds on uncultivated land.

*Soap as a Spreader.*—The addition of three or four pounds of soap (first dissolved in hot water) to each hundred gallons greatly increased the effectiveness of the arsenical sprays. This addition was found to be especially desirable when the sprays were used against the grasses or other waxy-coated foliage upon which the spray had a tendency to collect in drops.

#### ARSENIC ON WILD MORNING-GLORY

*Effect on the Soil.*—The germination and growth of the natural weed seeds on the plots after treatment has been used throughout



Fig. 9.—The effect of arsenic on the soil when applied by the leaf absorption method (see table 3).

A closer view of Spray Plot 1B. The luxuriant growth of weeds subsequent to spraying is good evidence of the non-injurious effect of arsenic on the soil when applied by the leaf absorption method.

the experiments as an indication of the effect of the treatments on the soil. The luxuriant growth of weeds shown in figures 8 and 9 is good evidence of the non-injurious effect of arsenic on the soil when applied by the leaf-absorption method. While arsenic has been shown to be very toxic to the soil when applied in large quantities, as in the root-absorption method, the amount required by the leaf-absorption method is so small that no ill effects to the soil could be detected. Even six successive applications of the spray failed to reveal any indication of deleterious effects.



A study of the effect of arsenic on the soil organisms that produce nitric nitrogen has been made by Professor W. F. Gericke, of the Division of Soil Chemistry and Bacteriology of this station. So far as this important function of soil fertility is concerned, this study has shown that an amount of arsenic equivalent to one hundred successive applications of the spray, as applied to the Centerville plots, produced no toxic effect on the nitrifying bacteria. It is evident, however, that the functions of the soil are interfered with when large quantities of arsenic are applied. In the root-absorption experiments, the above amount of arsenic rendered the soil barren of all vegetation, except morning-glory, for many months.

The writer is indebted to Professor Gericke for the following contribution concerning the effect of arsenic on the flora of the soil:

TABLE 6  
NITRIFICATION OF SOIL FROM CENTERVILLE  
Treated with sodium arsenite to kill weeds

Laboratory number	Plot number*	Depth of sample in inches	As <sub>2</sub> O <sub>3</sub> applied to plots in the form of sodium arsenite oz. per sq. yd.	Mg. of nitric nitrogen per 100 gms. soil		Average of determinations
				Duplicates (1)	(2)	
1	A-1	0-6	16	2.3	4.0	3.16
2	A-1	12-24	16	20.0	14.0	17.00
3	A-3	0-6	8	18.0	18.0	18.00
4	A-3	12-24	8	10.4	12.0	11.20
5	A-7	0-6	4	24.8	24.0	24.40
6	A-7	12-24	4	26.0	26.8	26.40
7	A-10	0-6	1	24.8	26.0	25.40
8	A-10	12-24	1	24.0	20.0	22.00
9	E-2	0-6	0	20.0	20.0	20.00
10	E-2	12-24	0	22.0	20.0	21.00

\* See table 1, page 73.

The data in table 6 show that the sodium arsenite applied at the rate of the equivalent of 8 ounces, or more, of arsenic trioxide per square yard was toxic to the soil organism that produced nitrification. Toxic effects were noted in both surface and subsoil in these larger applications. On the plots in which the sodium-arsenite application was equivalent to 4 ounces or less of arsenic trioxide per square yard, no toxic effect was noted; on the contrary, the results indicate stimulation of the soil organism that produce nitric nitrogen. The results are in accord with the data published by Greaves<sup>16</sup> who reported stimulating effects to ammonification and nitrification in soil to which small amounts of certain arsenic compounds were added.

*Concentration of Spray.*—Different concentrations of arsenical sprays were tried at Spreckels, varying from the equivalent of four ounces to sixteen pounds of arsenic trioxide per hundred gallons. The concentration of arsenic used throughout the Centerville experi-

ments (four pounds of arsenic trioxide per hundred gallons) appeared to be as effective for the control of morning-glory as any other tested. Much lower concentrations were ineffective. Higher concentrations appeared to produce no better results and possibly not as good. The stronger sprays in some instances appeared to collapse the tissues of the vines and stop the circulation of the sap before the poison had its full effect upon the roots.

*Repeated Applications of Small Doses.*—Repeated small doses of arsenic were sprayed upon morning-glory vines at intervals of one day, four days, and longer, depending upon the growth of the new vines. These experiments were tried in the spring of 1916 to ascertain if the effects of arsenic applied in small doses would be cumulative. None of these treatments, however, appeared to injure the roots in any way, although the experiments were continued well into the summer months.

*Absorbing Surface.*—The observation has been made many times that the roots of morning-glory plants without a good vine development (that is, with only a small absorbing surface) were not severely injured by the application of a poisonous spray to the vines; and that parts of the plants beneath the surface at the time of spraying would make a normal growth of vines subsequently, if not connected with a part which did have a good growth of vines. This observation contributes important evidence not only of the absorption of poisons through the aerial parts of wild morning-glory, but also of the non-injurious effect of the arsenical spray on the soil.

The broad-leafed weeds were generally more affected by arsenical sprays than the grasses. The effect of arsenic upon foliage (especially on grasses and other waxy-coated foliage) was more severe if soap was added to the spray to act as a spreader.

*The Time of the Year.*—The arsenical sprays, excepting a few very dilute ones, completely killed the vines of the wild morning-glory at whatever time of the year they were applied.

The earliest time at which any of these sprays were at all injurious to the roots was in May, at Davis, where the weed matured earlier than on any of the other experimental plots. At this time many of the plants were in the green seed-pod stage. All sprayings at Davis from May to October produced at least slight injury to the roots, apparently depending on weather condition, but none of them was considered a satisfactory control.

The spring and early summer sprayings on all of the other experi-

mental plots produced little or no injury to the roots of the weed, while the late summer and fall sprayings produced marked injury to the roots of mature vines. The amount of injury apparently increased as the season advanced. The most satisfactory results in 1915 were obtained in November; in 1916, in October.

The establishment of this point, however, was not a solution of the problem, as will be shown by later discussion.

*Maturity of Plants.*—Observations which appear to have an important bearing on the problem are as follows:

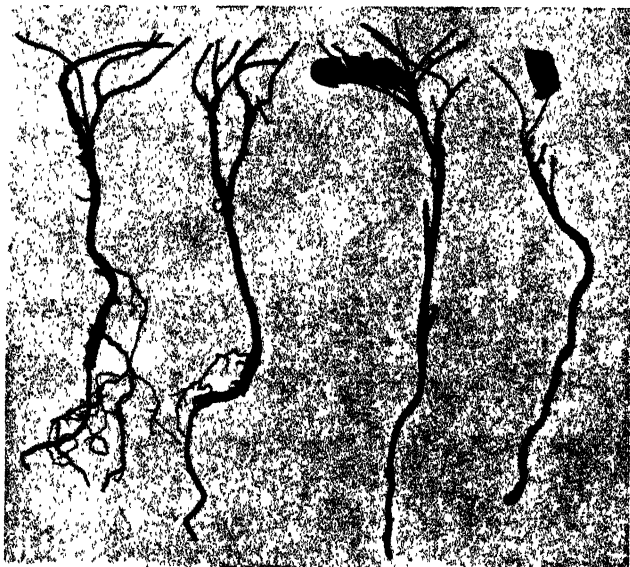


Fig. 10.—The maturity of the plant influences the results.

The roots of some of the morning-glory plants at Centerville were slightly injured by an arsenical spray applied June, 1916, and by one applied a month later. The roots of those plants which were in seed or late blossom at the time of spraying appeared to have been most severely injured. The same observation has been made repeatedly during the progress of the other experiments.

A spray was applied to mature morning-glory plants at Berkeley in September. The roots of these plants were completely destroyed by the spray. A spray applied to another plot, however, under practically the same conditions, failed to injure the roots. It was observed that in the latter case the vines were actively growing at the time of treatment, since they had been cut about four weeks previously with a weed knife. One plant, however, was found the roots of which were

moldy and disintegrated as far as dissected out. This vine apparently had been missed by the weed knife and was much older than the other vines. It had a number of green seed-pods attached, while the other vines had not yet blossomed. The two disintegrated roots shown at the left of figure 10 were taken from the plant mentioned above as being in seed at the time of spraying; the two uninjured roots at the right of the figure from which normal new sprouts had originated were taken from another plant only eight inches from the first. So far

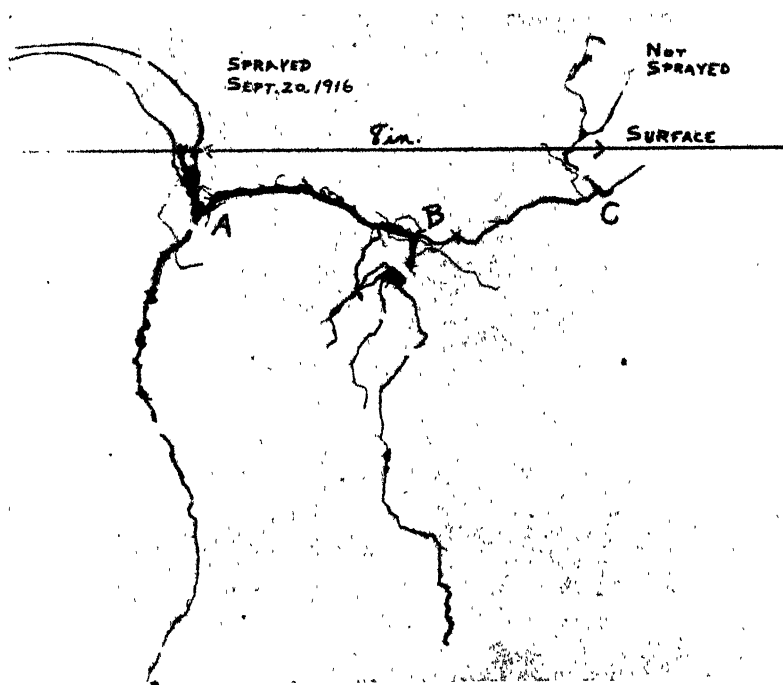


Fig. 11.— Typical morning-glory root system from the experimental plots on the campus at Berkeley.

The entire plant was in a state of disintegration when dug three weeks after spraying. The aerial parts originating from A were sprayed, while those originating from C were not sprayed. The vines originating from C were just beginning to wilt three weeks after the application of the spray above A.

as known, the general climatic conditions and local weather conditions at the time of spraying were practically the same at this point and on the plot on which the roots were entirely destroyed. Sprays were applied to mature vines at Berkeley many times during September and October, 1916, and in every case destroyed all signs of life in both vines and roots. Identical treatments in October at Centerville, at Spreckels, and in Ventura County resulted in very serious injury to the roots of the weed.

Another experiment which will throw light on the same matter was made at Davis. Two plots were selected, on one of which the plants were in full bloom and on the other in the green seed-pod stage. Identical sprays were applied to the two plots on the same day. Subsequent observations failed to reveal any injury upon the roots of the plants which were in full bloom at the time of spraying. The roots on the plot which was in the green seed-pod stage were seriously injured by the spray and fully 75 per cent of the roots failed to produce new sprouts within a month after the time of spraying. One root dissected out from this plot to a depth of three feet was found to be moldy and disintegrated throughout its entire length.

It thus appears that the most significant difference which may account for apparent conflicts of results was the difference in the stage of maturity of the plants. The remarkable phenomenon observed on the effect on the root systems of wild morning-glory vines produced by a late summer or fall spraying strongly suggests that the appearance of this phenomenon is intimately associated with the rest period of the plant. The increased downward flow of the sap of plants at the beginning of the rest period is well known to plant physiologists and appears to be a most significant fact in this connection.

*Influence of the Weather.*—Monthly climatological data for California have been received through the courtesy of the Weather Bureau of the United States Department of Agriculture. The reports from the weather station nearest the various experimental plots may be taken as indicating the approximate state of the weather at or near the time of treatment. In many cases the data were not sufficiently local or complete to determine accurately to what extent the results of the experiments were influenced by the weather. From a general survey of the weather data and the known facts concerning the influence of weather on foliage injury caused by insecticides and fungicides,<sup>14</sup> it seems evident that the most important weather condition influencing the results of the leaf-absorption method is the amount of moisture present at or near the time of treatment. The encouraging results obtained by the leaf-absorption method experiments in all the humid coast climates and the unsatisfactory results obtained by the same method in the semiarid climate at Davis strongly indicated that one essential condition of the successful application of arsenic as an herbicide by the leaf-absorption method is the presence of sufficient moisture to prevent the drying of the poison on the leaves before its absorption.

Although none of the experiments at Davis was wholly satisfactory, even though applied to mature vines which had already ripened seeds, two sprays were made in the fall which quite seriously affected the roots of the morning-glory vines. In both cases, the root injury occurred from the sprayings which were immediately followed by damp weather.

*Possibilities and Limitations of the Method.*—The method described can not as yet be said to be one of eradication. It has been demonstrated, however, that 85 to 90 per cent of the morning-glory roots on the plots near the coast can be killed to a depth of four feet or more by a properly timed spray to mature vines. New sprouts will emerge from the stubs of the partially killed roots and will eventually reach the surface and produce new vines. Under these conditions they are, however, very puny, the leaves being only about one-fourth of the normal size and of a sickly yellowish color. The new growth is quite different from the normal trailing vine. When the new shoot reaches the surface, a thick clump of erect branches is produced not more than eight or nine inches in length. The majority of the new shoots consume from seven months to one year in reaching the surface so that a crop can be well established on the land, or an early crop harvested, without serious interference from the weed.

The abnormal condition of the vines originating from the stubs of the partially destroyed roots and the enfeebled condition of these root stubs lead one to believe that an annual fall spraying may eventually eradicate the weed.

## SUMMARY

None of the root-absorption experiments seemed to point the way for the control of wild morning-glory on agricultural land at a reasonable expense and without serious injury to the soil.

Incidental to the main object of the experiments, the control of wild morning-glory on agricultural land, data have been obtained which show the superiority of arsenic as a soil sterilizer. All of the Centerville plots to which an ounce or more of arsenic trioxide had been applied per square yard were barren of all vegetation, except wild morning-glory, for fourteen months notwithstanding the leaching by the rains of two winters.

The leaf-absorption experiments have definitely established one fact of importance from a scientific as well as from a practical stand-

point, namely, that a dilute solution of sodium arsenite applied only to the aerial parts of the wild morning-glory under certain conditions will destroy both the aerial parts of the plant and the underground parts to a depth of several feet.

The experiments point strongly toward the conclusion that the accomplishment of these results is chiefly dependent on the coexistence of two conditions at the time of the application of the poison, namely that:

1. The plant is approaching or wholly within the dormant state.
2. Sufficient moisture is present in the air to prevent the drying of the poison on the leaves before its absorption.

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ON THE EXISTENCE OF A GROWTH-  
INHIBITING SUBSTANCE IN  
THE CHINESE LEMON

BY

H. S. REED AND F. F. HALMA

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SUBSTANCE IN THE CHINESE LEMON<sup>1</sup>

BY

H. S. REED AND F. F. HALMA

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It is the purpose of this preliminary paper to point out the probable existence of a growth-inhibiting substance or substances in the stem of Chinese lemon, a variety of the citron (*Citrus medica*), and to discuss some of its effects upon the development of shoots on the cuttings.

Vöchting<sup>2</sup> believed that each isolated stem is the carrier of a "force" which determines polarity.

The older botanists, under the leadership of Sachs, assumed that there was a flow of shoot-forming substances toward the apex and of root-forming substances toward the base in a regenerating cutting. The difficulty in this assumption, so far as the Chinese lemon is concerned, lies in the fact that none of the subapical buds develop. If the apical buds of the cutting develop as the result of the flow of elaborated materials in their direction, it is difficult to see why these materials which flow past subapical buds should not give them a stimulus to develop, though less in degree.

In a series of recent articles, Loeb has turned attention toward the possibility that there are one or more specific substances in the growing stem which determine the course of events in the growth of new shoots and roots in cuttings. He has shown that in *Bryophyllum calycinum* the apical bud prevents the lower ones from growing out, and he concluded that there is an inhibitory substance sent in the

<sup>1</sup> Paper no. 53, Univ. Calif. Grad. Sch. Trop. Agr. and Citrus Exp. Sta., Riverside, California.

<sup>2</sup> Vöchting, H., *Organbildung im Pflanzenreich*, Bonn, 1878.

direction of the basal buds. He<sup>3</sup> also believes that the reason why the apical bud grows out first is that it is the first bud which is freed from this substance when the stem is cut from the mother plant.

This view receives strong support from studies on the growth of potato sprouts recently published by Appleman.<sup>4</sup> He finds when all the buds on a tuber are subjected to the same external conditions, that the buds on the apical end grow out first, and that they inhibit the growth of the more basal buds. If these apical sprouts are destroyed, or retarded in their normal growth, sprouts will appear from the more basal buds which would otherwise have remained dormant. The cutting of a furrow around a middle or basal bud will cause it to grow out just as quickly as the apical buds of the tuber. If the tuber is cut into transverse slices the inhibitory influence of the apical buds is removed and there is a general growth of buds over the surface of the entire tuber, depending upon the thickness of the slices.

In the following experiments the cuttings were made from young wood of the Chinese lemon and varied in length from 15 to 40 centimeters. They were suspended in covered glass jars or in glass cases in which a moist atmosphere was continually maintained. The experiments were conducted in a culture room at a temperature which had a daily range of not more than one or two degrees centigrade. Illumination was obtained from two large incandescent electric lights fitted with filters of "Trutint" glass. The jars stood upon a revolving table which was driven at a speed of one revolution every three minutes by a small motor.

Shoots of the Chinese lemon which have attained a sufficient degree of maturity retain their vitality for many months after removal from the tree, provided they are kept in a moist atmosphere. The latency of the buds is not so easily broken as in the case of willow; they therefore constitute a more favorable subject for experimentation.

#### REGENERATION OF SHOOTS FROM CUTTINGS

When cuttings made from shoots of the Chinese lemon are suspended in a moist atmosphere, shoots develop from buds at the apex of the cuttings, and roots from the basal end. This occurs whether the cuttings are in the normal or inverted position (figs. 1, 2). The cuttings will grow for several months but no shoots will appear except from two or three buds nearest the apical end of the cutting. If the

<sup>3</sup> Loeb, J., The chemical basis of axial polarity in regeneration, *Science*, n.s., vol. 46, p. 547, 1917.

<sup>4</sup> Appleman, C. O., Physiological basis for the preparation of potatoes for seed, *Bull.* 212, Maryland Agr. Exp. Sta., 1918.

buds nearest the apex are killed by burning them with a hot glass rod at the time of installing the experiment then the buds immediately below them will develop, but if the apical buds remain functional and develop the lower buds remain dormant. If the sprout from an apical bud be removed after it has reached a length of one centimeter or more, a new sprout will develop from one of the accessory buds but the lower buds remain dormant. If the tip of a sprout be removed as in the left-hand cutting in figure 1, it will continue to grow from one of its lateral buds but the interruption of its growth does not cause the buds below it to develop.

Similar behavior is shown by shoots on the Chinese lemon tree itself. Figure 3 shows four upright shoots, averaging one meter high, which were taken from a Chinese lemon tree. A few lateral buds near the apex of each shoot had developed into short branches, yet the large number of buds below them had remained dormant.

This dominance of the buds nearest the apex of the shoots is so characteristic and so clearly marked that one can only conclude that it is due to some significant internal influence.

We may now turn our attention to some experiments designed to throw light upon the nature of this influence.

Cuttings about 30 centimeters in length were suspended vertically in a glass jar until one or two shoots were produced at the apex. A notch deep enough to remove bark and phloem was then cut immediately above several subapical buds and the cuttings replaced in the jar. After three weeks nearly every healthy bud above which a notch had been made either produced a shoot or showed signs of activity (fig. 1). The unnotched buds remained dormant. The same result was obtained when the cuttings were hung upside down (fig. 2). None of the buds on the control cuttings developed except those immediately back of the apex. The response is often more manifest if the sprout from the apical bud is allowed to reach a length of one or two centimeters before notching the subapical buds.

The next experiment shows what happens when the development of the apical buds is temporarily inhibited by mechanical means.

Cuttings about 30 centimeters in length, of as uniform size as possible, were selected and the upper half of some of them was enclosed in a plaster of Paris cast. They were then suspended vertically in a glass case and placed on the revolving table. In most cases the casts prevented development of the buds. After a little over three weeks some of the cuttings produced a sprout below the plaster cast.

Figure 5 shows two of these cuttings on which the new sprouts were 8 and 11 centimeters in length respectively. The sprout on each cutting was allowed to grow until it reached a length of several centimeters, then the plaster cast was removed and the cutting again suspended in the moist chamber.

The behavior of the cuttings released from the plaster cast was significant. Soon after removing the casts the apical buds of each cutting developed into sprouts in the normal manner. Figure 6 is from a photograph of the two encased cuttings shown in figure 5, and was taken one month after removing the casts. It shows that the shoots, which had previously developed, began to die when the apical buds began to grow. Subsequently the original shoots died, while those from the apical buds grew on normally. The result of this experiment shows two significant facts: (1) the shoots produced from apical buds after removing the casts were not inhibited in any manner by those already present near the middle of the cuttings; and (2), on the contrary, the original shoots near the middle of the cuttings were inhibited in their growth and finally killed when the apical shoots began to grow. The length of the sprout produced by the subapical bud might alter these relations, for example Mogk<sup>5</sup> reported that subapical sprouts in *Vicia faba* seedlings did not inhibit growth of the apical sprouts unless their relative lengths were as three to one.

The results of these experiments seem to us to indicate that there is some substance produced by the growing shoot which travels in the phloem layers toward the basal part of the stem and that it inhibits the development of buds situated nearer the base of the stem. In making a cutting such as that shown at the left in figure 1, we are isolating a piece of a stem whose buds, according to this assumption would have been previously prevented from developing by a substance emanating from the upper shoots or apical buds. If the substance which was included in this cutting remained evenly distributed throughout its length, none of the buds would have developed, but its tendency seems to be to migrate toward the basal end of the cutting.

The question might arise whether the results may not be due to a tendency for elaborated materials to pass upward more than downward, and to induce the development of apical buds only, through an accumulation of nutrients at the apical end. This question seems to be answered in the negative by two observations: (1) so far as known, the movement of substance in the phloem is downward; (2) the shoots

<sup>5</sup> Mogk, W., Untersuchungen über Korrelation von Knospen und Sprossen, Arch. f. Entwickelungsmech. d. Org., vol. 38, pp. 584-681, 1914.

produced from short cuttings having only one or two buds were as large and grew as fast as those produced from the first or second apical bud of a longer cutting.

It cannot be assumed, however, that the movement of this hypothetical substance is directed solely by the pull of gravity. As shown by the growth of cuttings such as illustrated in figure 2, it moves from apex to base even when the cutting is suspended inverted, in a vertical position. It would appear that the substance moves primarily in a basal direction, but that it is influenced to some extent by the pull of gravity.

The formation of new shoots on horizontal branches or on horizontally placed cuttings of Chinese lemon, affords some significant illustrations supporting the assumptions concerning the existence of a growth-inhibiting substance. The horizontal shoot on a tree behaves very differently from vertical shoots, such as those shown in figure 3. Whereas very few lateral buds develop on a vertical shoot, a great many buds develop on a horizontal shoot, provided they have reached a sufficient degree of maturity. Figure 4 shows a long shoot which originally grew vertically on the tree. So long as it was vertical none of the lateral buds developed into shoots. It was bent over and tied in a horizontal position. A few months later this photograph was taken and shows a development of lateral buds along nearly the entire length of the shoot. Shoots of the same age left in the vertical position remained like those shown in figure 3. Inspection of the branch will show, furthermore, that the buds which developed are those situated on the dorsal or upper side. Buds located on the lower side of the horizontal shoot did not develop. This was true of branches which grew naturally in a horizontal position, as well as of those which were bent into that position.

Cuttings suspended horizontally in glass cases produced new sprouts only from buds on the upper side, but not from the lower side (fig. 7). The sprouts were not confined to the apical end of the cutting, though they usually appeared first in that region. The growth of sprouts at the apical end exhibited no such signs of dominance over the other buds on the upper side of the cutting as was shown in the case of cuttings suspended vertically.

This development of the dorsal buds of a horizontal Chinese lemon shoot appears to be in conformity with the idea of a growth-inhibiting substance in the shoot. It appears that the substance accumulates on the lower side of the shoot and prevents its buds from developing,

at the same time freeing the upper side and allowing buds on that side to develop into new shoots.

A very striking result was obtained if a horizontal cutting was revolved through an arc of 180 degrees, so that the position of the upper and lower buds was reversed, after sprouts on the dorsal side had attained a length of 6 to 10 centimeters. A new sprout would soon appear from a bud upon what was previously the ventral side of the cutting. As soon as this new sprout began to grow, the original sprouts began to deteriorate and finally died (fig. 8).

It seems possible that the injury to the original sprouts might have been due both to the growth-inhibiting substance originally present in the cutting, and to additional material produced by the new sprouts. At any rate this substance appeared to accumulate at the lower side of the cutting and to inhibit growth in the shoots which had been brought into that position by the revolution of the cutting. It is important to notice that the two original sprouts on this cutting did not respond normally to the geotropic stimulus previous to their death. This would indicate that there were profound changes in the metabolism and in growth reactions of the sprouts.

It should also be noticed that the root which developed on the cutting shown in figure 8 showed no injury following the revolution of the cutting. It responded to its new position by curving and growing downward as before. The influence of this hypothetical substance upon root development is under investigation and will not be discussed here.

#### SUMMARY

1. Chinese lemon shoots produce very few lateral branches so long as they grow in a vertical position. Cuttings produce two or three new shoots from buds nearest their morphologically apical end. This dominance of growing buds at the apex of a shoot is characteristic and is also shown by inverted cuttings.

2. The dominant influence of the buds nearest the apex may be prevented from reaching lower buds by notching the phloem layers just above each bud. If the apical bud is prevented from developing by mechanical means, lower buds may develop.

3. Horizontal branches or cuttings of this tree produce lateral shoots only from the dorsal or upper side.

4. The theory is advanced that the shoots developing nearest the apex form a substance which is capable of inhibiting the growth of

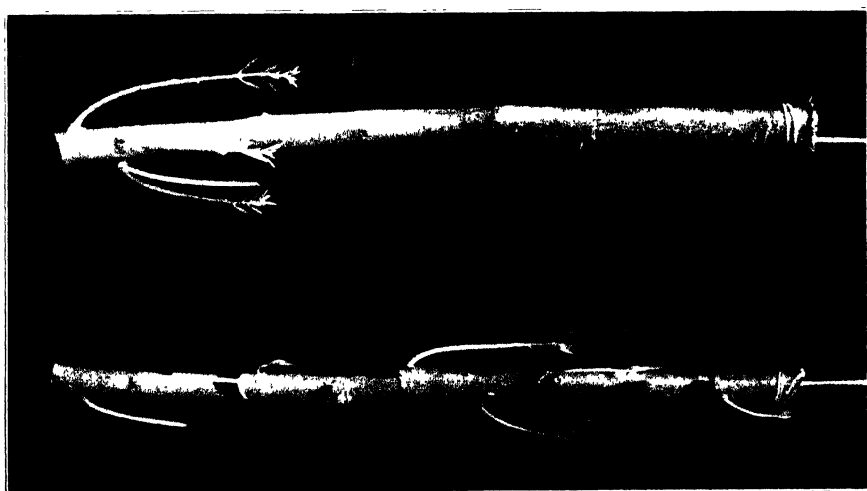
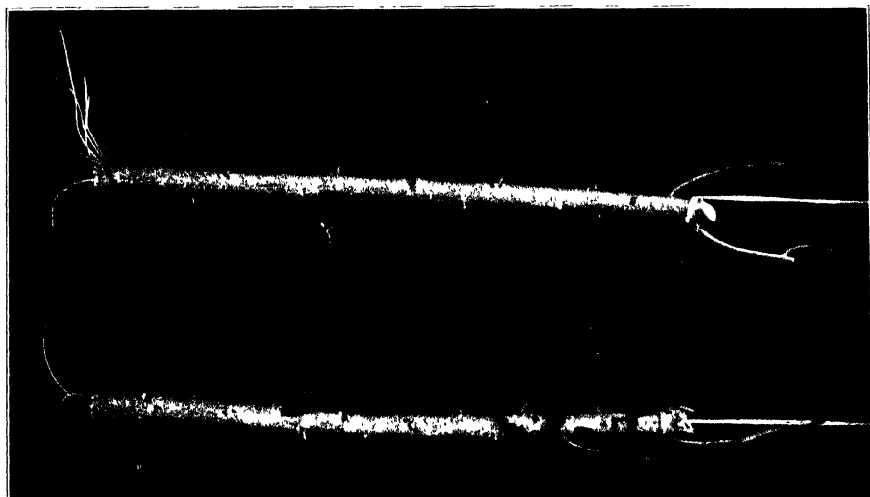
other buds on the vertical stem. The hypothetical substance appears to move toward the morphologically basal end of a vertical shoot or piece of shoot. It appears to have a strong deleterious effect upon growth and to perpetuate a condition of dormancy in subapical buds. In horizontally placed shoots this substance appears to settle to the lower side of the shoot.



PLATE 3

Fig. 1. Cuttings of Chinese lemon which had been suspended vertically. Cutting at the left produced two new shoots from the buds nearest the apex. Cutting at the right was notched just above several buds, some of which developed into new shoots.

Fig. 2. Cuttings of Chinese lemon, inverted and suspended vertically. Cutting at the left produced new shoots from buds nearest the apical end of the cutting. Cutting at the right was notched by removing bits of bark and phloem just above the buds. Most of the notched buds developed into new shoots.



#### PLATE 4

Fig. 3. Vertical shoots taken from a Chinese lemon tree. Each shoot has a few small branches shortly below the apex, but no others.

Fig. 4. Branch of a Chinese lemon tree which was bent horizontally from an originally vertical position. Buds on the upper side of the branch developed into shoots.



Fig. 3



Fig. 4

## PLATE 5

Fig. 5. Cuttings of Chinese lemon suspended vertically. The apical end of two cuttings was enclosed in a plaster cast. In each case a shoot was produced from the first healthy bud below the cast. Control cutting shown at the left.

Fig. 6. The two cuttings shown at the right of figure 5. Photograph made one month after removal of the casts. The subapical shoots shown in figure 5 died after new shoots developed from the apical buds.

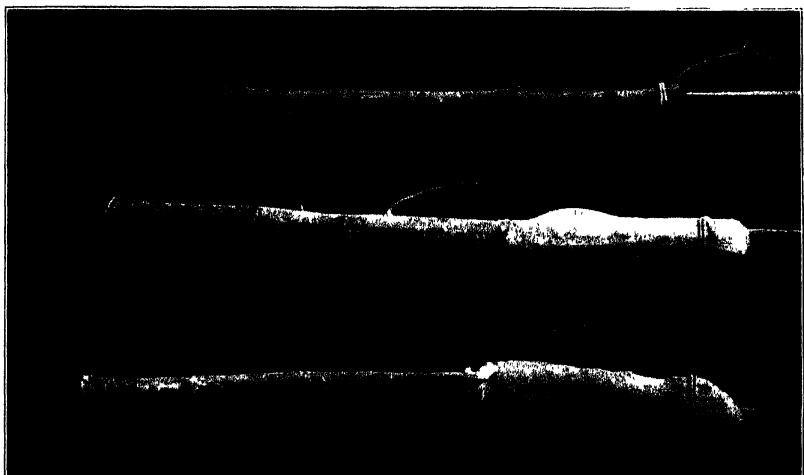


Fig. 5

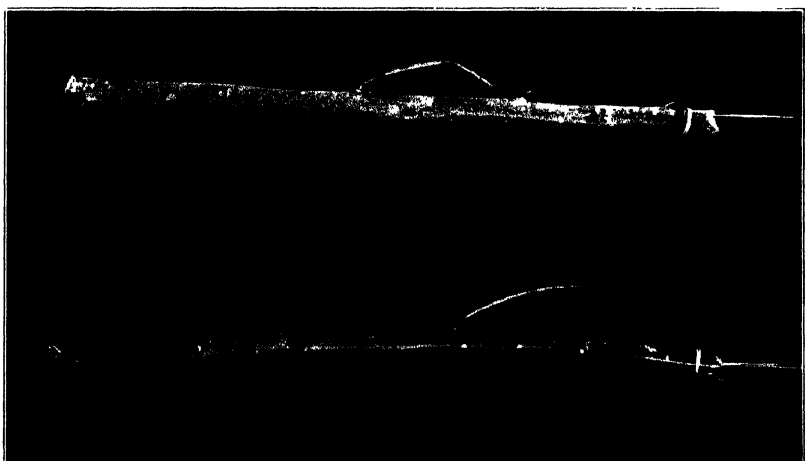


Fig. 6

## PLATE 6

Fig. 7. Cuttings of Chinese lemon suspended horizontally. Buds on the upper side developed into shoots.

Fig. 8. Cutting of Chinese lemon suspended horizontally. After it had produced two new shoots from the upper side the cutting was revolved through an arc of  $180^\circ$ , so that the position of the upper and lower buds was reversed. A new shoot was produced from what was then the upper side and the two original shoots died.

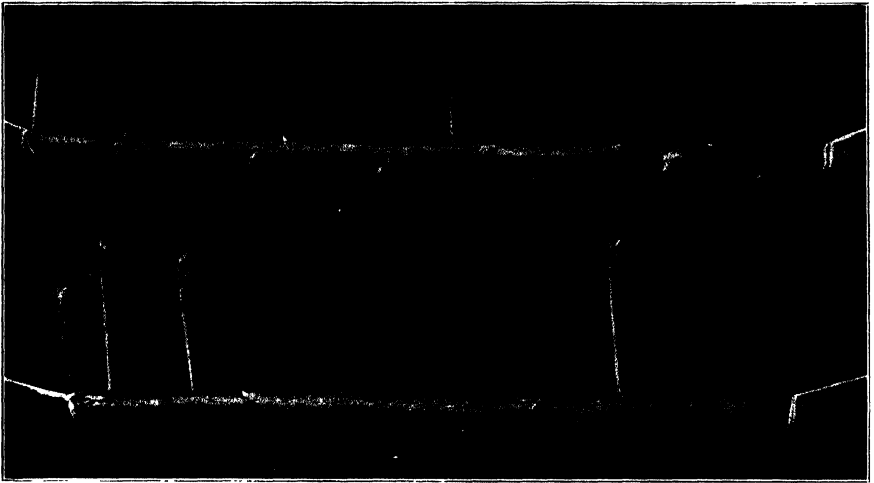


Fig. 7

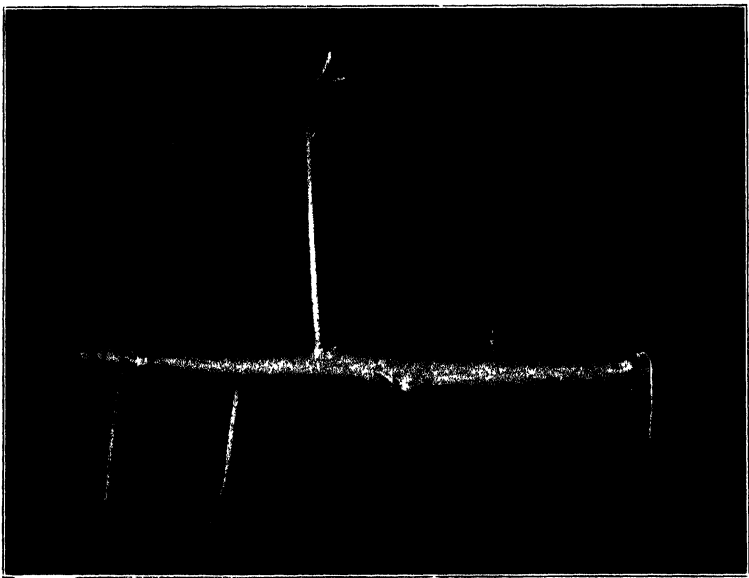


Fig. 8



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FURTHER STUDIES ON THE DISTRIBUTION  
AND ACTIVITIES OF CERTAIN GROUPS  
OF BACTERIA IN CALIFORNIA  
SOIL COLUMNS

BY  
CHARLES B. LIPMAN

UNIVERSITY OF CALIFORNIA PRESS  
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FURTHER STUDIES ON THE DISTRIBUTION  
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BACTERIA IN CALIFORNIA SOIL COLUMNS.

BY  
CHARLES B. LIPMAN

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More than six years ago I prepared for publication, and soon thereafter published<sup>1</sup> some results of certain of my studies on the bacteria of California "soil columns." In that paper I pointed out that by dint of the somewhat laborious Remy method I concluded that "arid" soils behave differently from "humid" soils in respect to certain groups of their bacterial flora, in that among other things active bacterial growth seems to go on at relatively great depths in the first class of soils and only at shallow depths in the second. Much has transpired since that statement appeared which has contributed to extensive changes in science in general and in soils science in particular. Positive facts and logic have been presented which have shaken to their foundations some of the most dearly cherished and firmly established concepts of a former generation. The fate of vast accumulations of data on soils hangs in the balance, if, indeed, their doom is not already sealed. All of that has perforce altered or is altering the objectives of soil work generally, and has exerted a profound influence on the determination regarding the validity and significance of the plans, purposes, and results of the investigations from which the present study emanated.

To introduce properly and to justify what follows, if such justification be needed, it may not be amiss to make brief reference to what might be termed the parent investigations from which the bacteriological studies in question sprang, and particularly of the

<sup>1</sup> Lipman, C. B., The distribution and activities of bacteria in soils of the arid region, Univ. Calif. Publ. Agri. Sci., vol. 1, pp. 1-20, 1912.

present status of their results and the effect of these results on the investigations described in this paper.

For many years, and especially during the last fifteen years of their lives, the late Professors Hilgard and Loughridge carried out mechanical and chemical analyses on what they termed the soil columns of California. These soil columns, which are now represented in their entirety in our collections, were series of soil and subsoil samples of what Hilgard and Loughridge regarded as the most typical soil classes in the state and particularly those typifying the arid soil conditions. To obtain these samples a type of post-hole auger, manufactured by Iwan Brothers, at South Bend, Indiana, was employed, with special extensions made for our use in boring at considerable depths. A sample, representing an average of every foot in depth, was usually taken down to and including the twelfth foot wherever ground water, thick hardpan, or similar obstacles did not interfere. If such obstacles were encountered the samples were taken as deeply as possible. Thus, while in most cases the columns represented twelve feet of soil in depth there were a number representing only four, six, or seven feet. The mechanical analyses were carried out by the Hilgard elutriator method, and the chemical analyses by the Hilgard strong-acid digestion method. The ultimate object of this work was to construct a map showing the important soil classes of California as then regarded, and, by giving information on the mechanical and chemical constitution of the soils, to enable the farmer to understand the physical and chemical suitability, or incompatibility of his soil for given crops, as well as their dependence on, or independence of fertilization.

To render his data more complete, and incidentally to throw some light on the microorganisms of arid soils, which at that time was practically an untouched field, Hilgard invited the writer more than a decade ago to begin a study of the bacteria of arid soils and to include in such study the soil columns of California. Recent studies in my laboratory, carried out by one of my associates, Dr. D. D. Waynick, have shown what we have been suspecting for the last four or five years, the hopelessness of Hilgard and Loughridge's plan. The reasons for this may be briefly stated as follows:

1. Soils are so markedly variable that a column collected in one spot cannot be considered representative of anything. Even a composite sample of truly representative nature is practically impossible to obtain.

2. A mechanical analysis gives no idea of the actual arrangement of the soil particles *in situ*; hence it is only the crudest kind of guide to the soil's physical characters.

3. The chemical analyses were carried out by an arbitrary method; but this in itself would not be so serious if any true correlation had ever been made between a soil's chemical composition and its crop-producing power. This has thus far not been done; besides, the analytical method in question was perhaps the least likely to figure in such a correlation if it should be attained. Moreover, the first point made above wholly negatives the validity and utility of analytical results obtained on such samples and by such methods as those under consideration.

Necessarily these fallacies were bound to affect the bacteriological studies, and many of the results as well as those of the chemical and mechanical analyses are now resting in the obscurity which they deserve. However, one outcome at least of the bacteriological studies possesses a scientific and perhaps also a practical value; that is, the determination of the depths to which microorganisms penetrate in arid soils and at which they are probably active. As pointed out in the opening lines of this paper, some of the results obtained in work on that problem have already been published. Further results would have been published if the method involved had not been so laborious. Recently, however, we have found by a careful investigation that for all practical purposes the auger-collected samples are just as good in every way as those collected by the special and laborious method which I devised ten years ago. The results of comparative tests of these two methods are given in a recent paper<sup>2</sup> by D. E. Martin and the writer. The fact that the auger-collected samples are just as reliable as those collected with special and great precautions, made possible the collection of ten more soil columns to a depth of six feet, inclusive. The results of studies on these additional soil columns form the chief topic of this paper, and are published primarily for the purpose of showing that bacteria and other microorganisms may penetrate to a depth of at least seven feet in most, if not in all arid soils. No other significance is claimed for the data submitted.

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<sup>2</sup> Lipman, C. B., and Martin, D. E., Are the usual precautions necessary in taking soil samples for bacteriological tests? *Soil Science*, vol. 6, no. 2, p. 131, August 19, 1918.

## PLAN OF PROCEDURE

The samples were taken by Mr. D. E. Martin with the Iwan post-hole auger. The detailed method of sampling is given in the paper cited above. The samples were shipped to the laboratory in tight Mason fruit jars. The locations at which the samples were collected are as follows:

*No. 1, Roseville.*—Three hundred yards due west of high school; fifty feet east of southeast corner of new city park; old grain field; Bureau of Soils classification, San Joaquin sandy loam. Decomposed hardpan, two feet to two feet six inches, and clayey material below.

*No. 2, Wheatland.*—One hundred and fifty yards southwest of Southern Pacific station; ten yards west of highway in young peach orchard, on edge of high ground. Bureau of Soils classification, Aiken fine sandy loam.

*No. 3, Gridley.*—One hundred yards northwest of Southern Pacific station; twenty yards north of main street; middle of block in vacant lot; water table at five feet. Bureau of Soils classification, Hanford sandy loam.

*No. 4, Marysville.*—One hundred yards east of highway; one-half mile south of Marysville viaduct; Yuba River bottomland; ten feet elevation; bean field; highly productive. Bureau of Soils classification, Columbia silt loam.

*No. 5, Grass Valley.*—From apple orchard; head of Auburn Street (north end), Grass Valley; residual from granite. Bureau of Soils classification, Sierra clay loam.

*No. 6, Davis.*—From University Farm at Davis. Bureau of Soils classification, Yolo silt loam.

*No. 7, Modesto.*—Twenty yards west of highway; one hundred yards north of warehouse Modesto Fuel Company; northern outskirts of town of Modesto. Fresno fine sandy loam, brown phase.

*No. 8, Fresno.*—Southeast corner of Harvey and Blackstone avenues, East Fresno; vacant lot. Bureau of Soils classification, Madera sandy loam.

*No. 9, San Gabriel.*—Ten yards east of San Gabriel highway; one-half mile north of junction with El Monte Road (Valley Boulevard); strawberry patch. Bureau of Soils classification, Hanford fine sandy loam.

*No. 10, Pasadena.*—Twenty yards west of Orange Grove Avenue; ten yards south of Pasadena Avenue (southwest corner); orange grove. Bureau of Soils classification, Placentia sandy loam.

The tests made on the soils consisted of the usual ammonification, nitrification, and nitrogen fixation determinations, with no pretense at attaching importance to the absolute values obtained. All the tests were made in the well known soil cultures in tumblers. For the ammonification tests, one gram of dried blood was mixed with fifty grams of soil and incubated for seven days under optimum temperature and moisture conditions. For the nitrification tests, one hundred grams of soils were used in every culture, the cultures being arranged in three ways, viz., soil alone, soil plus 1% dried blood, soil plus .2% ammonium sulphate. The incubation period was one month under the usual conditions of moisture and temperature. For the nitrogen fixation test, fifty-gram portions of soil were employed with 1% mannite. The incubation period was three weeks, at optimum moisture and temperature conditions. The results of these tests are given in the accompanying tables. Only averages of the determinations are given in the tables, for two reasons. In the first place, the duplicates agreed closely in most cases; in the second place, no special importance, as already indicated above, needs to be attached for our purposes to the absolute figures. For simplicity and convenience we shall discuss briefly each table separately.

#### THE AMMONIFICATION RESULTS

The data obtained and given in table 1 speak for themselves. There is every indication in them that ammonia-producing organisms, including both bacteria and fungi are active at considerable depths in all the soils. Indeed, there is little indication in our results that the ammonifying activities of the soils studied are inferior below six feet to those above six feet. That the uniformly high, bacterial efficiency at ammonia production is not the result of contamination of one soil layer by another had already been proved in the paper above cited,<sup>2</sup> and is proved again in the nitrification table accompanying this paper. In other words, we seem to be justified in accepting as definitely proved the fact that microorganisms, in arid soils do penetrate to considerable depths; particularly is this true of the ammonia-producing organisms, which in the soils here studied show about as great an efficiency in the sixth as in the first foot in depth.

### THE NITRIFICATION RESULTS

A study of table 2 reveals at once the great differences characterizing the ammonifying and nitrifying powers of the ten soils studied in this investigation, and especially below the first foot in depth. While it is impossible in table 1 to find indisputable evidence that the ammonifying power of any of the soils decreases downward from the surface foot, the evidence is more than ample in regard to nitrification in table 2. In practically every case the surface foot of soil is not only superior but usually far superior to those below it in efficiency at nitrate production. The contrast is indeed very striking. The data help, moreover, in proving that the method of sampling employed is justifiable and valid. Despite all this, however, table 2 gives unquestionable evidence in support of the idea that even nitrifying organisms do penetrate as far down as the seventh foot of soil under arid conditions. While their work may be feeble in many arid soils at considerable depths it is quite vigorous in many others at similar depths. Such activity is manifested regardless of the form of nitrogen available for nitrification. As a rule, it appears that the more fertile soils, like those at Gridley and Davis, are those in which bacterial activity is greatest in the deeper layers. Owing to the organic matter supply in such soils, contrasted with that in the poorer soils, like those at Fresno and Modesto, it is natural that the bacterial efficiency should vary accordingly, which, indeed, is just as true in the surface foot of soil as in the deeper layers.

### THE NITROGEN FIXATION RESULTS

The nitrogen fixation results are given in table 3. They cannot be regarded as being of much significance, owing to the considerable error which attaches to a nitrogen determination on a ten-gram portion of soil. The determinations were made in duplicate, but only the averages are given in the table; these represent five times the quantity of nitrogen found in ten grams of the soil culture, minus the amount found in the sterile control portion taken for analysis. It is easy to see that such manipulation may easily lead to serious errors. However that may be, the results are given for whatever interest and value they may possess. In the case of the San Gabriel soil, it is probably true that the figures represent actual gains in nitrogen, and it is interesting to note that while nitrogen fixation in



this soil is less in the lower than in the upper layers, it seems to be quite definite. From this soil, at least, it would appear that the nitrogen fixation results are in general accord with the ammonification and nitrification results regarding the chief point made in this paper, viz., that bacterial life does extend into the deeper layers of the soil under arid conditions. Through an oversight the Pasadena soil was not studied in regard to nitrogen fixation, hence no statement appears with regard thereto in table 3.

### SUMMARY AND CONCLUSION

From studies on twelve soils, two of which are discussed in another paper and ten in this paper, the writer has been able to confirm his findings of several years ago to the effect that microorganisms of arid soils penetrate deeply into the subsoil layers.

In the present paper it has been demonstrated that ammonifying vigor continues undiminished through six feet of soil in every case. While this is not true for the nitrifying powers of the same soils, it is clear that nitrifying bacteria do live in the lower layers of some, and perhaps of all of the soils to similar depths. The great difference between the two, however, lies in the fact that the nitrifying power of these soils diminishes rapidly downward from the first foot while the ammonifying power remains apparently unchanged. The nitrogen fixation results are for the most part inconclusive, but such as are above question seem in general to confirm the idea that bacteria and other microorganisms do penetrate to greater depths in arid soils than one would expect. Such penetration and activity at those depths seem to be superior to those noted on humid soils, so far as we have evidence in the literature upon which to base such a comparison.

TABLE 1.—AMMONIFICATION  
Milligrams of Nitrogen as Ammonia Produced

Name of soil	Depth in feet					
	1	2	3	4	5	6
Roseville .....	30.84	31.89	29.17	10.00	22.12	20.79
Wheatland .....	35.84	39.13	32.60	28.27	28.00	22.96
Gridley .....	38.22	32.20	36.96	30.80	22.82	14.49
Marysville .....	33.81	34.79	35.28	38.65	36.33	36.96
Grass Valley ....	23.10	24.50	20.30	22.96	23.94	21.63
Davis .....	68.18	54.88	44.94	37.66	40.60	41.58
Fresno .....	38.71	38.64	37.03	36.54	33.74	30.52
Modesto .....	38.71	42.63	40.18	39.48	42.14	41.40
San Gabriel .....	42.14	38.57	50.22	38.36	37.24	32.34
Pasadena .....	56.35	52.64	46.34	52.71	41.93	18.18

TABLE 2.—NITRIFICATION  
Milligrams of Nitrate Nitrogen Produced per 100 Grams of Soil

Name of soil Treatment	Depth in feet					
	1	2	3	4	5	6
	Soil N	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	Dried blood	Soil N	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	Dried blood
Roseville	1.00	2.00	0.10	0.60	0.80	0.10
Wheatland	3.00	6.00	16.00	1.50	2.30	13.50
Gridley	12.80	128.00	25.60	3.20	6.80	8.20
Marysville	4.10	22.00	25.50	2.20	12.50	6.00
Grass Valley	5.90	20.50	11.00	2.00	8.60	2.00
Davis	6.00	12.10	16.60	4.30	10.50	19.50
Fresno	1.90	2.00	0.10	0.60	0.40	0.10
Modesto	0.70	0.80	0	0.60	0.60	0
San Gabriel	3.00	6.00	1.00	2.00	6.00	0
Pasadena	1.20	20.00	1.30	1.00	4.00	0
	Soil N	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	Dried blood	Soil N	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	Dried blood
	0.20	0.10	0.10	0.20	0.20	0.10
	0.60	0.80	0.60	0.60	0.70	0.80
	1.60	12.80	1.50	1.30	9.80	2.20
	1.30	2.20	1.80	1.50	9.30	0.80
	0.30	0.60	0.60	0.20	0.40	0.60
	2.00	3.80	0.20	1.90	6.10	0.40
	0.40	0.50	0	0.40	0.40	0
	0.20	0.20	0	0.30	0.40	0
	0.40	0.70	8.00	0.70	2.50	8.00
	1.00	0.60	0.20	0.60	1.50	0.20
	0.60	0.40	0.40	0.60	0.60	0.40

TABLE 3.—NITROGEN FIXATION  
Milligrams of Nitrogen Fixed per Gram of Mannite

Name of soil	Depth in feet					
	1	2	3	4	5	6
Roseville	.35	—1.75	6.65	5.25	2.25	1.90
Wheatland	—1.40	—	.35	—1.05	—2.80	1.05
Gridley	1.05	.70	—	.35	—	.35
Marysville	—8.75	2.10	1.05	.35	1.05	—
Grass Valley	5.70	.70	2.10	.70	—	.35
Davis	6.65	9.45	11.45	—	.70	—1.75
Fresno	—	3.15	4.20	.70	2.10	3.15
Modesto	1.75	.35	2.10	1.05	—	.35
San Gabriel	15.40	9.10	11.90	5.60	1.75	.35

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May 10, 1919

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II. VARIATIONS IN NITROGEN AND CARBON IN FIELD  
SOILS AND THEIR RELATION TO THE  
ACCURACY OF FIELD TRIALS

BY

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UNIVERSITY OF CALIFORNIA PRESS  
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SOILS AND THEIR RELATION TO THE ACCURACY  
OF FIELD TRIALS

BY  
D. D. WAYNICK AND L. T. SHARP

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INTRODUCTION

Investigations carried out under field conditions are subject to a number of variables, all, or any one of which may serve to invalidate the results which may be secured. The problem of adequate experimental control is an exceedingly difficult one, and unlike many experiments carried out in the laboratory none of the variables met with in the field are capable of complete elimination. They may, however, be allowed for, at least in so far as they are concerned with the soil mass, if we know the magnitude of the variables met with. The heterogeneity of the soil, even in experimental plots, has been quite generally recognized as a factor which may render the data secured from field plots open to more or less serious errors; but that the soil may be so variable as to render the measurements made of soil constituents of very questionable value has not been fully appreciated. There are considerable data, gathered at various places, from which very definite conclusions have been drawn without first making allowance for the variables entering into the problem in hand. Unless a high degree of certainty exists that the results secured in the first trial will be obtained when the trial is repeated the experimental data are of little value.

The problem of variability in experimental trials with field crops has formed the subject of a number of recent papers.<sup>1</sup> It is not our purpose to enter into a discussion of the problem of the control of field experiments from the crop standpoint. It is proposed, however, to consider the possible magnitudes of field variation in soils and the bearing of such variation on field trials in which the soil mass is the predominant factor. One of us has already taken up this subject from the standpoint of nitrate production in soils,<sup>5</sup> and since no data are obtainable which show the magnitude of the variation in some of the constituents commonly measured in field soils it is of very considerable interest to present data which have been obtained from the viewpoint of the accurate control of the soil as a variable factor. It must be understood in considering the data presented in the following pages that the variations found and the statistical constants computed are by no means absolute figures which can be taken bodily and applied to any soil, but rather that they will serve to indicate the extent and nature of the variations likely to be found in field soils, together with the methods which it is hoped will prove of value in allowing for such variations as are found in any soil. The point has previously been made that variations in the soil within very limited areas may be so great as to render results secured in the past, with only a few samples taken from the area, of very limited value. The data obtained, since the first paper was written, and presented in this and the following papers make this viewpoint all the more secure.

The present data were secured in connection with and preliminary to a field study of biologic nitrogen fixation now being conducted by this laboratory, and though incidental to that problem their bearing upon the results which may finally be secured is so important as to render their consideration from that viewpoint of much interest. This is equally true of field experiments of a similar nature which have been, or are being conducted elsewhere, and is the principal reason for the presentation of the following data at the present time.

## METHODS

Two fields are concerned in the present study, one on the University Farm at Davis and the other near the town of Oakley. The soils of these two fields are of very different character, a silty clay loam at Davis, and a blow sand at Oakley. The total area sampled in each

field is a little more than one and three-tenths acres. The fields were both selected for their apparent uniformity, both being nearly level, with no changes in the soil mass from one part of the field to another great enough to be detected by the usual field methods. Both fields were practically free from vegetation when selected, and before the samplings were made (March, 1918) all extraneous material had been carefully removed. Since the data presented in the previous paper were the only data which would serve as any sort of a guide to the number of samples necessary to secure the degree of accuracy desired, and since it was necessary, further, to distribute the samples in such a manner as to cover the entire area, the arrangement shown in figure 1 was adopted. It will be noted that there are eighty samples distributed at thirty-foot intervals over the entire area, forty samples at fifteen-foot intervals taken from five different parts of the field, these also uniformly distributed, and finally twelve samples taken within one of the small areas, approximately one-forty-eighth of an acre, in the center of the field. There are, then, one hundred samples from each area under consideration. The entire number of samples will be treated as one population, except in so far as they are useful in showing the effect of the distance apart samples are taken upon the variability, and, further, the relationship of the variability in a given small area within a field to that of the entire field. It is recognized that the data available at the present time on this last point are limited, due to the small number of samples available, but they are at least suggestive of the relationship between the two.

All the samples were taken uniformly with a three-inch soil auger by foot sections. As soon as taken each foot section was thoroughly mixed and approximately one-half of the sample placed in a quart jar of the Mason type. When all the samples had been secured the jars were placed in specially constructed boxes and shipped by express to the laboratory at Berkeley. All the samples were there reduced to the air-dry condition as rapidly as possible and passed through a two-millimeter sieve. The determinations, as herein reported, were made upon the mass of soil passing through a sieve of this size.

Total nitrogen was determined on ten-gram samples of the Davis soil, or twenty-gram samples of the Oakley soil, using the modification of the Kjeldahl-Gunning method proposed by Hibbard.<sup>2</sup> Eight hundred cubic centimeter Pyrex flasks were used in making all the determinations, so that it was not necessary to transfer to copper flasks for the final distillation. All the titrations were made with

standard hydrochloric acid, 1 c.c. of which was equal to .54 milligrams of nitrogen. Determinations for total carbon were made upon five and ten gram samples, respectively, of the two soils by a modification of the wet combustion method described by one of us elsewhere.\*

#### LIMITS OF ACCURACY OF METHODS FOR NITROGEN AND CARBON

It is not proposed to take up a discussion of the accuracy of experimental methods in general. Before experimental work with field soils is undertaken the accuracy of the laboratory methods to be used in the investigation should be known. This is true because, in the first instance, any factors tending toward inaccurate results from the laboratory standpoint are possible of elimination to a large extent, and, in the second instance, the limitations of the laboratory methods may finally define the limits of accuracy of results obtained in the field. In the present study, all errors which may be termed accidental have been eliminated so far as possible by careful attention to the details of manipulation.

That determinations made upon the different portions of the same sample may still differ considerably among themselves is shown by an inspection of tables 1 and 2, where a number of determinations for nitrogen and carbon are reported, all made upon the same soil sample. The results presented here are of further interest in showing how nearly values found for a composite sample may conform to the mean of the individual samples making up that composite. The latter point will be considered more at length later.

TABLE 1.—VARIABILITY OF DETERMINATIONS FOR TOTAL NITROGEN MADE UPON A UNIFORM SOIL SAMPLE

No.	Nitrogen per cent	Deviation from mean	No.	Nitrogen per cent	Deviation from mean
1	.092	.004	16	.096	.000
2	.096	.000	17	.093	.003
3	.097	.001	18	.097	.001
4	.099	.003	19	.096	.000
5	.094	.002	20	.097	.001
6	.098	.002	21	.101	.005
7	.092	.004	22	.098	.002
8	.099	.003	23	.097	.001
9	.100	.004	24	.099	.003
10	.097	.003	25	.096	.000
11	.098	.002			
12	.097	.001	Mean =	.0960 ± .00034	.002
13	.092	.004	$\sigma$ =	.0025 ± .0002	
14	.096	.000	C. V. =	2.60 ± 0.24	
15	.099	.003			





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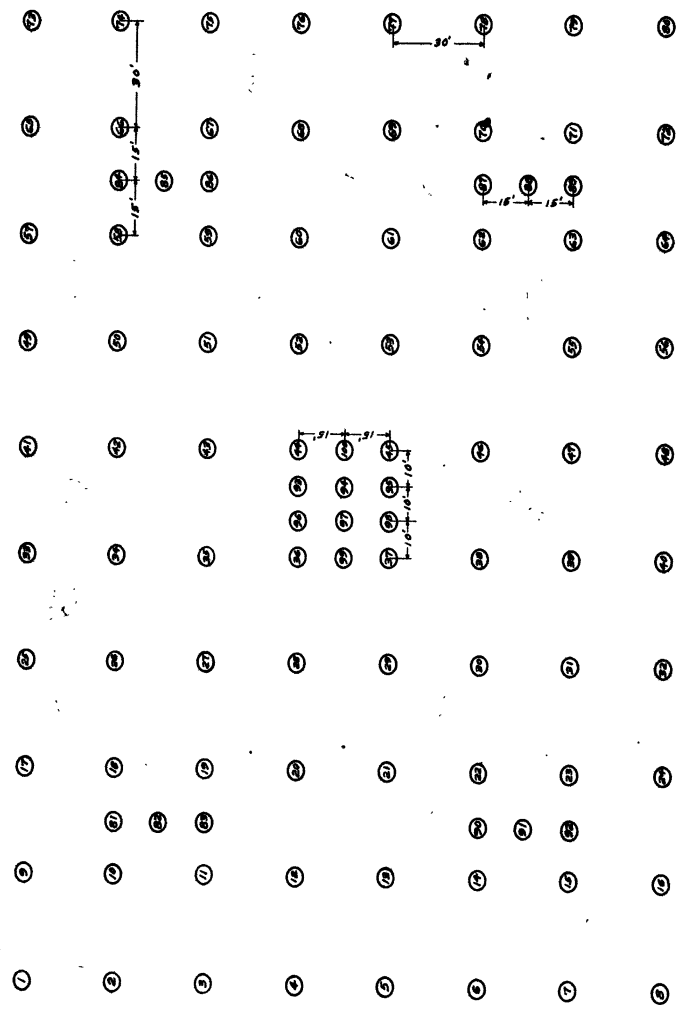


Fig. 1 Diagram of areas sampled, showing the locations from which the samples were taken.

TABLE 2.—VARIABILITY OF DETERMINATIONS FOR TOTAL CARBON MADE UPON A UNIFORM SOIL SAMPLE

No.	Carbon per cent	Deviation from mean	No.	Carbon per cent	Deviation from mean
1	1.093	.012	11	1.081	.000
2	1.076	.005	12	1.093	.012
3	1.077	.004	13	1.076	.005
4	1.092	.011	14	1.073	.008
5	1.065	.016	15	1.082	.001
6	1.092	.011	16	1.069	.012
7	1.091	.010			
8	1.091	.010			
9	1.080	.001			
10	1.071	.010			
				Mean = 1.081 $\pm$ .0015	.008
				$\sigma$ = .009 $\pm$ .001	
				C. V. = .83 $\pm$ .09	

If a number of determinations are made upon a given sample and the values so secured are arranged according to the frequency of their occurrence it will be found that they group themselves after the manner of a normal error curve. This fact has already been discussed in connection with the determination of nitrates by the colorimetric method and is introduced here because it serves as a background for the consideration of limits of accuracy of the methods under discussion. Since a series of results such as given in table 1 or 2 do follow a normal error curve, we are justified in using the statistical constants calculated from them as a measure of the accuracy of our methods. We have, for example, in the case of nitrogen, the mean of twenty-five determinations, expressed as  $.0960 \pm .0003\%$ . This mean is, then, probably correct to within  $\pm 0.31\%$  of the actual amount of nitrogen determined. If, however, but a single determination had been made it would have been subject to a probable error of  $.0960 \pm .0016\%$  of  $\pm 1.66\%$  of the amount of nitrogen found. On the other hand, the probable error of the mean of one hundred total nitrogen determinations amounts to  $\pm .00016\%$ , or  $\pm .016\%$  of the nitrogen present. It is evident, therefore, that the larger the number of determinations made upon any given sample the greater will be the accuracy of the mean of the determinations made, but only in proportion to the square root of their number. It is possible on this basis to determine the amount of nitrogen present in any soil with a very high degree of accuracy, provided a sufficient number of determinations can be made. This conception is derived from the fact that what we have chosen to term the laboratory error is purely a matter of chance. Therefore, a series of these errors, whether derived from several determinations on a single sample or from individual determinations on different samples, if plotted by classes, would be dis-

tributed as a normal frequency curve in accordance with the laws of chance. Computations of such a series of errors would yield the same statistical constants whether the errors were from the determinations made on a single sample or were secured from single determinations made on separate samples. This point is of importance, and unless recognized before any determinations are made upon soil taken from any experimental area, many determinations may be made which will, in the final analysis, lend nothing to the final accuracy of the results. This is true when we are dealing with the mean of a representative number of determinations. The worker may carry through his determination in duplicate or in triplicate, but such a practice will only serve to satisfy him as to the accuracy of his own manipulation, as such replications are of no value when a statistical interpretation of the data as a whole is made.

That the laboratory error is small when compared with the variation found in field samples is shown by the following data, which have been computed on a "pounds per acre" basis, using 4,000,000 pounds as the weight of one acre foot of soil. If twenty-five samples are taken the error which may be attributed to laboratory manipulation amounts to plus or minus twelve pounds per acre, with an even chance that our results are accurate to this amount. If we adopt a thirty-to-one chance as the largest chance which we deem it safe to take in order that our results will have a high degree of accuracy, the lowest significant figure amounts to plus or minus thirty-eight pounds per acre. In other words, with twenty-five determinations increases or decreases in the nitrogen found must exceed this figure before they reach a magnitude sufficiently great to be attributable to any treatment which we have applied. On the same basis, the mean of our total number of one hundred samples is probably accurate to within twenty pounds per acre so far as the laboratory error itself is concerned. However, when we consider that the error due to the variations in field samples amounts to two hundred and forty pounds per acre on an even chance, or seven hundred and sixty pounds on a thirty-to-one chance, it is seen that the laboratory error of twenty pounds becomes relatively insignificant. When we are dealing with the number of samples used in the present study, together with the relatively large amount of nitrogen determined, in the case of the Davis soil at least, it is evident that this laboratory error may be safely disregarded. It is recognized, however, that there may be instances in which the laboratory error may limit to a large degree

the accuracy to be expected from the use of a given number of field samples. This might be true, for example, in a case in which the total amount of nitrogen or any other element determined is so small that the laboratory error becomes relatively of large magnitude. It must always be borne in mind, however, that the curve representing the laboratory errors and the one representing the errors due to sampling are always parallel; that is, the two variables always decrease in the same ratio as the number of samples is increased.

The above discussion has been limited to a consideration of the probable accuracy of the nitrogen determination, and it has been treated somewhat at length because it is considered important that there should be a clear understanding of the limitations of the methods employed. The same mode of treatment might be followed in discussing the limits of accuracy of the carbon determination. These results will not be taken up in detail. Suffice it to say that the laboratory error attributable to our method may be taken as amounting to seventy-two pounds per acre on a thirty-to-one chance, while the mean of one hundred samples has a probable error, on the same basis, due to field variability, of approximately eight hundred pounds per acre.

We have deemed it important to take up a discussion of the limits of accuracy of the methods used in order that a false estimation of the accuracy of the measurements made may not be assumed. Many determinations have been reported in the past giving increases or decreases in the constituents commonly measured in field soils without consideration of the accuracy of the methods employed. For example, the amount of nitrogen removed by an average crop of barley is usually stated as about forty pounds per acre. We have shown that by making twenty-five determinations on a single soil sample, we are able, due to our laboratory errors, to determine nitrogen in amount not less than plus or minus thirty-eight pounds per acre, which is practically the same figure as given for the nitrogen removed by a single crop. It is evident, therefore, that we are just able to determine the amount of nitrogen removed by a single crop from determinations made upon the soil when the soil mass has been made as uniform as it could be made experimentally and a large number of determinations carried out on a soil so prepared. In other words, if we ignore the variability of the field samples and consider only our laboratory error it is found that the value for the nitrogen removed by a single crop and that for the error due to laboratory manipulations

is of nearly the same magnitude. When we take into account as well the errors due to sampling in the field, it is evident that the differences which must be secured before we can feel confident that the results are reliable are very much larger.

When the results of past soil investigations are examined from this viewpoint it will be found that most of them have been obtained under conditions so imperfectly controlled or under variations in sampling so inadequately taken into account, as to render the results obtained of very questionable value.

#### EXPERIMENTAL DATA

The experimental data for the two areas are reported in tables 3 and 4. It will be noted that the extreme range for nitrogen in the Davis soil is from .077 to .124% and in the Oakley soil from .022 to .063%, a difference between the extremes of about 60% in the first soil and nearly 300% in the second. If we translate these figures into a "pounds per acre" basis, we have a range of from 3100 to 5000 pounds in the first soil and from 900 to 2500 in the second soil. For carbon the range is from 0.903 to 1.383% in the Davis soil and from 0.252 to 0.750% in the Oakley soil, a percentage difference between the extremes of nearly the same magnitude as that given for nitrogen. Expressed on the acre basis in the same manner as the nitrogen figures given above, we have 36,000 to 55,000 pounds and 10,000 to 30,000 pounds respectively for the two soils. If but a single sample were taken at random to represent these two areas, it can be readily seen that a very inaccurate estimation of their nitrogen and carbon content might be made. Further, it is probable that these differences between samples taken from apparently uniform areas are quite as large as any that may be found between different soil types. In fact, the figures here presented are extremely interesting from that viewpoint, in that, while it may be contended that different soil types, as usually determined, have a range of values for total nitrogen, for instance, not covered by another soil type, unless a large number of samples are taken, it would be out of the question to distinguish between two soil types on that basis. For example, in the two soils under consideration, which are nearly as widely separated as soil types can be, one a silty clay loam and the other a blow sand, the difference between the high value for nitrogen in one and the low value for nitrogen in the other amounts to only .014%, or less than a third of the extreme range in

either of these soils. This is a very small value when we take into consideration the variability of the soil as a whole. In this connection, it is again emphasized that we are dealing with two very limited areas, selected because of their apparent uniformity.

It will be noted that the coefficients of variability for both nitrogen and carbon are 9.00% in the Davis soil, while in the Oakley soil they are much in excess of this figure, being 21.87% for nitrogen and 21.11% for carbon. It will be recognized that the coefficients of variability for the Davis soil as regards the two constituents here measured are low, so that this area may be properly considered as possessing a relatively high degree of uniformity. Just how true this is as regards other constituents will be brought out in a subsequent paper. The figures as here given may be used again to emphasize the statement made in the introduction of this paper to the effect that values found for one soil may be very misleading if an attempt is made to apply them as an aid in forming a judgment of the probable variation to be found in another soil.

#### EFFECT OF DISTANCE ON VARIABILITY OF SAMPLES

It has already been stated that because of the locations used in sampling it is possible to arrange our data in such a manner that we may calculate the statistical constants for any number of samples taken at intervals of thirty, fifteen or ten feet. The samples taken

TABLE 3.—TOTAL NITROGEN AND CARBON IN VARIOUS SAMPLES OF THE DAVIS SOIL

No. of sample	Nitrogen per cent	Carbon per cent	No. of sample	Nitrogen per cent	Carbon per cent
1	.104	1.167	18	.099	1.048
2	.086	1.048	19	.086	0.965
3	.080	0.958	20	.093	1.040
4	.092	1.069	21	.092	1.014
5	.099	1.071	22	.105	1.122
6	.098	1.132	23	.123	1.329
7	.107	1.124	24	.107	1.075
8	.117	1.195	25	.096	1.048
9	.105	1.059	26	.088	0.978
10	.093	1.084	27	.100	1.091
11	.086	1.077	28	.106	0.998
12	.091	0.997	29	.105	1.147
13	.082	0.896	30	.104	1.112
14	.105	1.198	31	.110	1.154
15	.114	1.218	32	.115	1.244
16	.108	1.157	33	.093	0.997
17	.094	0.970	34	.103	1.052

TABLE 3.—(Continued)

No. of sample	Nitrogen per cent	Carbon per cent	No. of sample	Nitrogen per cent	Carbon per cent
35	.101	1.045	71	.106	1.168
36	.106	1.019	72	.116	1.365
37	.103	1.120	73	.077	0.977
38	.113	1.215	74	.090	1.161
39	.110	1.202	75	.095	1.048
40	.111	1.167	76	.093	1.123
41	.093	1.007	77	.101	1.267
42	.091	0.973	78	.104	1.107
43	.096	1.100	79	.106	1.186
44	.100	1.135	80	.116	1.289
45	.097	1.052	81	.103	1.108
46	.096	1.034	82	.091	0.986
47	.104	1.090	83	.078	0.903
48	.116	1.252	84	.091	1.161
49	.093	0.993	85	.094	1.000
50	.109	1.180	86	.096	1.042
51	.107	1.126	87	.099	1.288
52	.102	1.153	88	.107	1.352
53	.106	1.256	89	.115	1.232
54	.106	1.250	90	.103	1.109
55	.118	1.254	91	.112	1.251
56	.124	1.383	92	.116	1.322
57	.101	0.973	93	.102	1.088
58	.090	1.001	94	.098	0.989
59	.101	0.973	95	.095	1.997
60	.101	1.120	96	.097	1.093
61	.098	1.051	97	.098	1.114
62	.098	1.101	98	.099	1.063
63	.107	1.195	99	.097	1.064
64	.101	1.254	100	.103	1.092
65	.086	1.088			
66	.103	1.137			
67	.088	1.009			
68	.094	1.078			
69	.100	1.173			
70	.097	1.071			

Mean = .100 ± .006	1.110 ± .007
A. D. = .006	.083
$\sigma$ = .0090 ± .0004	.1000 ± .0005
C. V. = 9.00 ± 0.42	9.00 ± 0.42

TABLE 4.—TOTAL NITROGEN AND CARBON IN VARIOUS SAMPLES OF THE OAKLEY SOIL

No. of sample	Nitrogen per cent	Carbon per cent	No. of sample	Nitrogen per cent	Carbon per cent
1	.042	.624	10	.032	.420
2	.032	.417	11	.024	.330
3	.027	.365	12	.024	.361
4	.022	.179	13	.031	.349
5	.029	.355	14	.032	.314
6	.026	.383	15	.033	.475
7	.027	.279	16	.042	.330
8	.031	.404	17	.042	.499
9	.042	.565	18	.028	.314



TABLE 4.—(Continued)

No. of sample	Nitrogen per cent	Carbon per cent	No. of sample	Nitrogen per cent	Carbon per cent
19	.027	.402	63	.032	.466
20	.021	.428	64	.034	.450
21	.037	.421	65	.045	.647
22	.022	.308	66	.028	.384
23	.036	.345	67	.030	.480
24	.034	.345	68	.031	.362
25	.041	.524	69	.035	.498
26	.029	.377	70	.033	.407
27	.025	.382	71	.039	.577
28	.029	.450	72	.031	.431
29	.034	.453	73	.061	.947
30	.026	.292	74	.040	.589
31	.027	.345	75	.041	.612
32	.031	.450	76	.040	.636
33	.042	.507	77	.063	.750
34	.027	.314	78	.036	.478
35	.026	.341	79	.040	.587
36	.028	.252	80	.035	.516
37	.035	.319	81	.029	.368
38	.027	.346	82	.025	.361
39	.026	.368	83	.022	.329
40	.031	.524	84	.035	.409
41	.052	.450	85	.032	.558
42	.030	.314	86	.030	.384
43	.031	.396	87	.036	.380
44	.028	.381	88	.032	.497
45	.032	.502	89	.033	.436
46	.029	.404	90	.024	.312
47	.030	.480	91	.033	.396
48	.032	.382	92	.028	.366
49	.046	.426	93	.027	.407
50	.031	.718	94	.026	.338
51	.033	.456	95	.029	.314
52	.032	.488	96	.027	.360
53	.034	.443	97	.029	.396
54	.033	.450	98	.037	.435
55	.034	.516	99	.029	.382
56	.033	.352	100	.029	.347
57	.052	.742			
58	.034	.461		Mean = .032 ± .005	.440 ± .007
59	.031	.475		A. D. = .007	.009
60	.031	.534		$\sigma$ = .0070 ± .0003	.115 ± .005
61	.031	.420		C. V. = 21.87 ± 1.03	25.11 ± 1.13
62	.032	.458			

at the last two distances were not uniformly distributed over the entire area but represent smaller areas within the larger. They are none the less useful in forming an estimate of the relation which a

small area may bear to a larger area within which it is located, or, as an interrogation, would we be justified in applying the value obtained for nitrogen in a plot of  $\frac{1}{50}$  of an acre to that in one acre? In the case of the Davis soil we may answer this question in the affirmative, since there is no significant difference between the mean of amounts of nitrogen found in the total population as related to the samples taken at either the fifteen or ten foot intervals as shown in table 5. With the carbon determinations the differences are slight. It will be noted, however, that the coefficients of variability of the samples taken at ten foot intervals is only about one-third as large as those found for the total population. With the Oakley soil, on the other hand, there is a significant difference in the means for both total nitrogen and total carbon for the samples taken at fifteen and at ten foot intervals. In other words, in dealing with a soil as variable as regards nitrogen and carbon as the Oakley soil we would not be justified in using the data obtained from a small area in the interpretation of that secured from the larger. The coefficient of variability of the samples taken at shorter intervals is significantly less than that calculated from the total population, although the difference between the two is less than half the coefficient of variability calculated for the total population in either case.

These data emphasize again the point which was brought out in the previous paper, that it is very unwise to judge an area even of the size reported here by values obtained from a limited portion of that area if the variations are of the magnitude reported for the Oakley soil. Estimations so made may be far from the truth and if extended in their application may lead to very erroneous conclusions.

#### ACCURACY OF VALUES SECURED BY USING COMPOSITE SOIL SAMPLES

From the standpoint of the amount of labor involved in making determinations in the laboratory the compositing of a number of individual samples is very desirable. Such a practice is permissible, however, only when the variations amongst individual samples in the area under consideration are known. When a larger area is broken up into plots the compositing of a number of samples taken in a representative manner from a small plot becomes almost necessary, since otherwise the amount of work involved in making the determinations on a large number of individual samples would be almost prohibitive. To test this point, the determinations reported in tables 1 and 2 were made on a composite sample, prepared by taking twenty grams from each of the samples of the Davis soil, numbered from 2 to 26 inclusive.

TABLE 5  
SHOWING THE RELATION OF SOIL SAMPLES TAKEN OVER SMALLER AREAS AND AT SHORTER DISTANCES APART TO A LARGER AREA  
AND TO THE TOTAL NUMBER OF SAMPLES TAKEN

	Total population		15-foot intervals		10-foot intervals	
	Nitrogen	Carbon	Nitrogen	Carbon	Nitrogen	Carbon
<i>Davis</i>						
Mean	$.100 \pm .006$	$1.110 \pm .007$	$.100 \pm .006$	$1.106 \pm .007$	$.099 \pm .007$	$1.069 \pm .010$
$\sigma$	$.0090 \pm .0004$	$.0100 \pm .0005$	$.0080 \pm .0008$	$.010 \pm .001$	$.0030 \pm .0006$	$.0450 \pm .0009$
C. V.	$9.00\% \pm .42\%$	$9.00\% \pm .42\%$	$8.00\% \pm .80\%$	$9.03\% \pm .95\%$	$3.53\% \pm .68\%$	$4.20\% \pm .81\%$
<i>Oakley</i>						
Mean	$.032 \pm .005$	$.440 \pm .007$	$.030 \pm .004$	$.382 \pm .006$	$.029 \pm .010$	$.368 \pm .012$
$\sigma$	$.0070 \pm .0003$	$.115 \pm .005$	$.0040 \pm .0004$	$.062 \pm .006$	$.0030 \pm .0006$	$.062 \pm .001$
C. V.	$21.87\% \pm 1.03\%$	$25.11\% \pm 1.13\%$	$13.33\% \pm 1.40\%$	$16.23\% \pm 1.71\%$	$11.72\% \pm 2.27\%$	$16.85\% \pm 3.26\%$

Just how closely the results agree is shown in table 6. In the case of both nitrogen and carbon the probable error in the difference between the two results is approximately equal to the difference between the two results, hence the latter value is of no significance.

It must be remembered, however, that we have made a large number of determinations on the composite sample, so that we have secured a much higher degree of accuracy than if but one or two determinations had been made. The making of a composite sample is, therefore, a justifiable procedure from the standpoint of the final accuracy of the results secured; provided, of course, that enough determinations are made upon the sample so that their mean will accurately represent the soil mass, and, further, that the composite itself represents equal amounts of the various individual samples which have themselves been made as uniform as possible by thorough mixing.

TABLE 6.—SHOWING THE RELATION BETWEEN DETERMINATIONS MADE UPON INDIVIDUAL SAMPLES AND ON A COMPOSITE SAMPLE MADE FROM THE VARIOUS INDIVIDUAL SAMPLES

	Mean		Probable error of mean	
	Nitrogen	Carbon	Nitrogen	Carbon
Determinations from composite sample Nos. 2-26 inclusive .....	.0960	1.081	.0003	.0015
Determinations on individual samples Nos. 2-26 inclusive .....	.0940	1.075	.0015	.0058
Difference .....	.0020	.006	.0015	.0059

#### NUMBER OF FIELD SAMPLES NECESSARY TO SECURE THE DEGREE OF ACCURACY DESIRED

We may adopt as the limit of accuracy to be secured in any investigation a value below which we attach no significance to our results. The limiting values which may be taken will depend largely upon the anticipated significance to be given to the results secured from the experiment in hand. Further, the physical capacity of the laboratory carrying on the experimental work will in many instances be the factor limiting the number of samples and thus the probable accuracy secured. When it is realized that the results obtained even by the most careful laboratory procedure, carried out with a larger number

of samples than heretofore used, may still be in error to such a degree that only large increases or decreases in soil constituents may be measured with accuracy, the importance of using as many samples as the capacity of the laboratory will permit becomes evident.

To emphasize the importance of a correct estimate of the number of samples necessary to measure certain changes in carbon and nitrogen which may take place in the two soils under discussion, the values given in table 7 have been calculated after the manner outlined in a previous publication.<sup>5</sup> In the second column the differences which we desire to measure are expressed on a "pounds per acre" basis, while in the third column these differences are given on a basis of percentage of soil, allowing 4,000,000 pounds per acre foot. In the

TABLE 7.—ESTIMATE OF THE NUMBER OF SAMPLES REQUIRED FOR ACCURATE MEASUREMENT OF CERTAIN CHANGES IN NITROGEN AND CARBON

	Limits of accuracy pounds per acre	Limits of accuracy percentage basis	Odds 1-1	Odds 10-1	Odds 30-1
<i>Davis Soil</i>					
Nitrogen	25	.0006	100	250	317
	50	.0012	25	62	79
	100	.0024	6	15	19
	250	.0062	.9	2	3
	500	.0125	.2	1	1
Carbon	25	.0006	12222	30555	38743
	50	.0012	3055	7637	9684
	100	.0024	764	1910	2425
	250	.0062	114	285	361
	500	.0125	28	70	88
<i>Oakley Soil</i>					
Nitrogen	25	.0060	61	125	193
	50	.0012	15	37	47
	100	.0024	4	10	12
	250	.0062	.6	1	2
	500	.0125	.1	1	1
Carbon	25	.0006	16685	41712	52891
	50	.0012	4170	10452	13218
	100	.0024	1042	2605	3303
	250	.0062	156	390	494
	500	.0125	39	97	123

next three columns is stated the number of samples necessary to take so that their mean will have a probable error no greater than that given in columns two and three. The number of samples required is listed under three headings, depending upon the chances we are willing to take that our experimental results can be duplicated under

the same conditions. With odds of one to one, the chances are even that our experimental results are capable of duplication. With odds of ten to one or thirty to one the chances are that in one hundred trials ten and three of them, respectively, will not give the same results as our original experiment.

It will be noted that if we accept twenty-five pounds per acre as our limiting value for nitrogen we must take no less than 317 samples from the Davis soil or 193 samples from the Oakley soil with odds of thirty to one. With carbon to be determined, no fewer than 38,743 and 52,891 samples, respectively, of the two soils must be taken. It is at once evident that these samples are too numerous from the standpoint of maintaining the integrity of the plot we are sampling or the capacity of the laboratory to handle the samples. We must, therefore, content ourselves with a lower degree of accuracy than twenty-five pounds per acre. As we increase our limiting value the necessary number of samples decreases very rapidly, so that with a value of about one hundred pounds of nitrogen per acre we require but nineteen samples of the Davis, or twelve samples of the Oakley soil. For the investigation in hand we have taken eighty pounds of nitrogen and eight hundred pounds of carbon per acre as our limiting values, which makes it necessary to use twenty-eight samples for nitrogen in the Davis soil and nineteen in the Oakley soil, with forty-eight and forty-one samples, respectively, for carbon. These values were chosen because this number of samples is within the capacity of the laboratory to make the determinations when we take into consideration the number of different plots under treatment. It is true, then, that when the investigation is finished, we will know that our results will not, with odds of thirty to one, have a probable accuracy greater than the figures given above. With the chances but even that we may be able to repeat our experiment and obtain the same results as secured when the trial was originally carried out the work involved in carrying through the original experiment is scarcely justified. Even odds of ten to one leave a large chance for error when attempts are made to repeat the experiment. We are hardly justified in taking a larger chance than thirty to one, for our experimental results will still probably fail of replication three times out of every hundred trials. Some field experiments may not justify the work involved in sampling to secure the degree of certainty desired in the present experiment. This may be so if the results so secured are to have a very limited application or are of a preliminary nature. In

general, however, it is felt that a larger chance than the one taken in this experiment is hardly justified and may only lead to further confusion in our interpretation of data secured through field experimentation.

It will be noted that fractional values are given in four instances. They are given only as the results of calculation as the taking of a fraction of a sample is obviously impossible. It must be remembered, however, that in using a small number of samples the laboratory error may be great enough to invalidate the results secured unless a sufficient number of determinations is made to reduce this error to a low value.

By the use of the method suggested for the calculation of the number of samples necessary to secure any desired degree of accuracy, two ends are attained. In the first place, the sampling of field soils becomes not something to be done in a haphazard sort of way, but rather a definite, practical procedure, the results of which may be predicted with a high degree of certainty. In the second place, much needless work may be avoided where more samples are taken than are really necessary to ensure the degree of accuracy desired.

As we have already stated before, we do not think our data sufficiently extensive to warrant a definite statement on the relation between the total area reserved for experimental purposes and small subdivisions made from that area. In general, it is probably true that small areas are less variable than the larger areas within which the former are located. That this may not always hold true is shown by the data for the nitrate found in the field soil reported in a previous paper, and, to a large extent, holds true for the data presented in this paper for the Oakley soil. It is hoped that as our work progresses with these two experimental plots we may secure data which will be of value in interpreting the relation of small areas to larger, and from such data we may likewise be able to correct, to a certain extent, for the heterogeneity in field soils after the contingency method proposed by Surface and Pearl.<sup>3</sup>

## SUMMARY

Data are presented in the present paper showing the variations found in total nitrogen and total carbon in two experimental areas, located upon soils of widely different types. Statistical methods are applied to the interpretation of the results obtained. The following statements seem justified from the results secured:

1. The extreme variations between different samples in which nitrogen and carbon were determined are of very considerable magnitudes and show that the results secured with one or a few samples would be likely to be unreliable.

2. It is unwise to attempt to apply the statistical constants calculated for one area to other areas even though in themselves apparently uniform since the respective variabilities may be very different.

3. Data are presented showing the making of a composite sample to be fully justified after the variations in the area to be sampled are known.

4. The relation of variations in a small area to differences between soil types, based upon the constituents determined is discussed, and the conclusion is drawn that only after very careful sampling may such differences be determined with certainty.

5. The advantages of estimating the number of samples necessary to secure any degree of accuracy are discussed. It is shown that a higher degree of certainty in experimental work may be secured by so doing, and also that in some instances needless labor may be eliminated.

In the past many erroneous conclusions have been drawn from data secured in field experiments with soils, which it is hoped may be avoided in the future by the application of the principles herein outlined.



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THE EFFECT OF SEVERAL TYPES OF IRRIGATION  
WATER ON THE PH VALUE AND  
FREEZING POINT DEPRESSION OF  
VARIOUS TYPES OF SOILS

BY  
D. R. HOAGLAND AND A. W. CHRISTIE

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# THE EFFECT OF SEVERAL TYPES OF IRRIGATION WATER ON THE $P_H$ VALUE AND FREEZING POINT DEPRESSION OF VARIOUS TYPES OF SOILS

BY

D. R. HOAGLAND AND A. W. CHRISTIE

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A number of years ago it became one of the duties of this laboratory to examine numerous samples of water with the object of deciding whether or not they were suitable for irrigation purposes. Although various investigations on the toxicity of alkali salts have been carried out, it was soon realized that no experimental data were available which afforded any adequate basis for the interpretation of the results of analyses of alkali waters. Ordinarily sufficient consideration has not been given to the very important principle that the soil and water form a chemical system and that effects on plant growth must be correlated with modifications in the concentration and composition of the soil solution. Conclusions concerning the latter cannot be deduced from the composition of the irrigation water alone; in fact it is as necessary to consider the soil as the water. This opinion has recently been stated clearly by Kelly.<sup>8</sup>

When the experiments described in this paper were begun, it was the intention to make a rather extensive investigation of the effects of alkali waters on soils, but other projects have prevented the carrying out of this plan. It is thought worth while, however, to place on record the data which have been obtained, since these are based on several new methods of study not heretofore applied to similar problems except in a few instances. It is hoped that the present brief discussion may be of some value to other investigators who may plan more comprehensive researches on the effects of alkali waters in their relation to the chemistry of the soil and the nutrition of the plant.

The principal methods which have been employed by the writers include the determination of OH ion concentration by the hydrogen electrode and of total concentration in the soil solution by means of the freezing point depression, as described by Bouyoucos and McCool.<sup>2</sup> These methods have been thoroughly discussed elsewhere, and it will suffice now to state that the degree of alkalinity in the sense of OH ion concentration can only be studied at all accurately with the aid of the hydrogen electrode or by some equivalent method; ordinary titration procedures do not yield the necessary information. With regard to the total concentration, the freezing point depression affords the most direct method of determining this value for the soil solution (the free water of the soil with a total moisture content suitable for plant growth). For purposes of comparison, there are also included in the present investigation data obtained by analyzing soil extracts prepared by mixing one part of soil with five parts of water. In addition, conductivity measurements have been made on 1:1 extracts.

The waters used in the experiments were made in the laboratory by dissolving pure salts in distilled water. The composition of the artificially prepared waters was based on six different types, selected from data given in bulletins of the United States Geological Survey. After long standing in glass containers analyses of the waters were made, as they were used in the experiments. The analyses have been calculated in terms of characteristic groups as proposed by the United States Geological Survey.<sup>10</sup> This seems to be the most logical and convenient method for classifying waters with regard to those properties which are of interest to the geologist or agriculturist. Since this scheme of classification has not been employed in publications of agricultural experiment stations, it may be desirable to mention here that the reaction coefficient for any ion is the factor obtained by dividing the valence by the atomic weight. This factor multiplied by the concentration of the element in parts per million gives the reaction value. By this system of equivalents all ions are placed on the same basis and subsequent calculations are greatly simplified. The characteristic groups mentioned represent the percentage properties of the water and are independent of concentration. Thus different waters may be classified readily in this way irrespective of total concentration.

In table 1 are presented the analytical results on the six waters used, as well as their percentage content of the various groups of ions. In total salt content they range from a water of considerable purity

TABLE 1  
COMPOSITION AND PROPERTIES OF WATERS

No.	Sodium* Na		Calcium Ca		Magnesium Mg		Carbonate CO <sub>2</sub>		Bicarbonate HCO <sub>3</sub>		Chlorine Cl		Sulfate SO <sub>4</sub>		Silicate SiO <sub>2</sub>		Total Solids	Percentage Characteristics			
	P.P.M.	Reaction value	P.P.M.	Reaction value	P.P.M.	Reaction value	P.P.M.	Reaction value	P.P.M.	Reaction value	P.P.M.	Reaction value	P.P.M.	Reaction value	P.P.M.	Reaction value	P.P.M.	Primary Salinity Na-SO <sub>4</sub> -Cl	Secondary Salinity Ca-Mg-SO <sub>4</sub> -Cl	Primary Alkalinity Na-CO <sub>3</sub> -HCO <sub>3</sub>	Secondary Alkalinity Ca-Mg-CO <sub>3</sub> -HCO <sub>3</sub>
A	48.1	2.09	17.5	.87	65.4	5.38	9.6	.32	124.4	2.04	7.1	.20	273.3	5.68	3.8	.10	540	25.1%	45.4%	none	29.5%
B	525.3	22.85	3.0	.15	2.5	.21	331.2	.11	34.2	.56	149.0	4.20	299.2	6.23	45.6	1.20	1390	45.0	none	53.5	1.5
C	473.8	20.61	7.0	.35	24.2	1.99	69.6	2.32	951.6	15.60	60.3	1.70	107.0	2.23	41.8	1.10	1260	17.1	none	72.7	10.2
D	27.4	1.19	5.0	.25	19.7	1.62	none	none	87.8	1.44	3.5	.10	70.0	1.45	2.5	.07	234	39.0	44.5	none	16.5
E	189.6	8.25	7.5	.38	5.0	.41	24.0	.80	417.2	6.84	35.5	1.00	15.6	.32	2.5	.07	504	14.7	none	76.6	8.7
F	378.0	16.44	45.5	2.27	7.9	.65	none	none	22.0	.36	613.4	17.30	80.2	1.67	1.3	.03	1256	85.0	13.0	none	2.0

\*Calculated.

to several of rather high concentration. None of the waters, however, has a concentration of salts as high as that frequently found in naturally occurring waters.

It is necessary at this point to make a brief statement with regard to the terms "alkali" and alkalinity, since these may often be used in different senses. The term "alkali" is generally employed to designate a condition of high total salt content in a water or soil and subclasses are referred to as "black" and "white" alkali, without any very definite or consistent line of demarcation. In this paper the expression alkalinity is applied to that condition in the soil or water which is characterized by a higher concentration of OH ion than of H ion. This condition is the resultant of an equilibrium into which many factors may enter, such as carbonates, bicarbonates, dissolved CO<sub>2</sub>, silicates, organic matter, etc. The two determinations then which are principally considered in this discussion are alkalinity as just defined and total concentration of salts. There is, however, no intention of giving the impression that other factors, such as the ratio of one element to another, may not be of great importance, but these questions cannot be answered with the data now at hand.

In the field the effect of an irrigation water on the soil solution will depend upon the total mass of soil reacting with the water, and upon the proportion of the products of the reaction not leached out. Thus the question of drainage is necessarily involved, and this is obviously a variable dependent on topographical conditions, soil texture, and the nature of the underlying strata. In the laboratory it is ordinarily practicable to study only the chemical effects of a given quantity of water on a definite mass of soil. In the present instance drainage effects are not considered, consequently the results may be interpreted as representing maximum effects in certain directions.

The first experiment was carried out with the use of three-gallon jars, in each of which were placed twenty pounds of air dry soil. Four different soils and a sample of beach sand were included, as shown in the tables. The first application consisted of two liters of water. After the soils had dried out, further applications were made, one liter at a time, until a total of seven liters had been added to each jar. The jars were kept outdoors, protected from rain, for a period of seven months. In the second experiment smaller quantities of soil were used. These were placed in large shallow covers of pottery, and water was added as in the first case, except that finally twice as much water was used in proportion to the soil. Two new

soils, as well as pure silica sand, were included in this experiment. In both experiments the dried out soils were mixed and samples preserved in bottles.

It is of course impossible to compare exactly the quantities of water used with those employed in irrigation practice, since we should have to assume some maximum depth of penetration. In the first experiments the quantity of water added to the soil would be approximately equivalent to fourteen inches per acre, if all remained in the first foot of soil; in the second experiment about twenty-eight inches were used.

Hydrogen ion concentrations were determined with a hydrogen electrode apparatus, as described by Sharp and one of the authors.<sup>13</sup> Ten grams of dried soil were mixed with 30 c.c. of water. Within wide limits the proportion of soil to water did not appear to effect the H ion concentration very greatly. To a certain extent at least the soil mass, carbonates, silicates, etc., form a "buffer" system. It is of course impossible to state with certainty whether the OH ion concentration of the soil solution would be the same as that found in the extracts. According to Plummer,<sup>11</sup> in acid soils a greater concentration of H ion is found in the film water. It is conceivable that in alkaline soils a greater OH concentration might be found in the soil solution. It seems highly probable, however, that the general magnitudes measured are fairly representative of the soil solution. The effect of carbon dioxide must also be considered. As already pointed out elsewhere,<sup>7</sup> in certain alkaline soils, if a sufficient concentration of CO<sub>2</sub> is maintained in the atmosphere in contact with the soil, there may result a marked reduction in the OH ion concentration. It is unlikely, however, that most soils of high alkalinity would be capable of maintaining such a high CO<sub>2</sub> production, though the addition of large quantities of organic matter might tend to bring about such a condition. With regard to the freezing point depressions, these were made on the soils at optimum moisture content, and so should give an idea of the concentration actually existing in the soil solution.

The main results of the experiments are shown in tables 2 to 7. The first item for consideration is the OH ion concentration. These concentrations are expressed in the customary P<sub>H</sub> values, as given in the tables prepared by Schmidt and one of the authors.<sup>12</sup> Neutrality is indicated by 7.0 and higher values denote increased concentrations of OH ion. Directing attention first to the three waters (B, C, E)



TABLE 2  
EFFECTS OF ALKALI WATER "A" ON VARIOUS SOILS

Description of Soils	Analyses of 1:5 Water Extracts*											
	P.P.M. Air Dry Soil											
	Total Solids		Bicarbonate HCO <sub>3</sub>		Chloride Cl		Sulfate SO <sub>4</sub>					
	Specific Resistance (25°C.) 1:1 Water Extract		Freezing point depression at optimum moisture content		pH Soil Suspension		Un- treated soil		7 liters water per 9 kilos soil		14 liters water per 9 kilos soil	
	Un- treated soil	7 liters water per 9 kilos soil	14 liters water per 9 kilos soil	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
Clay adobe No. 1 .....	8.7	8.9	...	...	...	...	...	...	...	...	...	...
Clay adobe No. 2 .....	7.0	...	7.1	.213	.396	.210	.126	.213	.396	.210	.126	.213
Silty clay loam No. 3...	7.2	7.3	7.1	.060	.064	.060	.060	.060	.064	.060	.060	.060
Fine sandy loam No. 4...	7.0	7.6	7.8	.077	.050	.077	.077	.133	.050	.077	.077	.133
Fine sandy loam No. 5...	7.0	...	7.5	.031	...	.031	.031	.118	...	.031	.031	.118
Beach sand No. 6 .....	6.5	7.1	7.2	.035	.102	.035	.035	.180	.102	.035	.035	.180
Silica sand No. 7 .....	6.2	7.0	8.4	...	...	...	...	...	...	...	...	...

\*The analyses of the water extracts of the untreated soils are omitted, since only negligible quantities of Cl and HCO<sub>3</sub> were present, except in the case of Soil 1.



TABLE 4  
EFFECTS OF ALKALI WATER "C" ON VARIOUS SOILS

Description of Soils	Analyses of 1:5 Water Extracts									
	P. P. M. Air Dry Soil									
	Total Solids		Bicarbonate HCO <sub>3</sub>		Chloride Cl		Sulfate SO <sub>4</sub>			
pH Soil Suspension	Freezing point depression at optimum moisture content		Specific Resistance (25°C) 1:1 Water Extract							
	Un-treated soil	7 liters water per 9 kilos soil	14 liters water per 9 kilos soil	Un-treated soil	7 liters water per 9 kilos soil	14 liters water per 9 kilos soil	Un-treated soil	7 liters water per 9 kilos soil	14 liters water per 9 kilos soil	14 liters water per 9 kilos soil
pH	Un-treated soil	7 liters water per 9 kilos soil	14 liters water per 9 kilos soil	Un-treated soil	7 liters water per 9 kilos soil	14 liters water per 9 kilos soil	Un-treated soil	7 liters water per 9 kilos soil	14 liters water per 9 kilos soil	14 liters water per 9 kilos soil
	pH	pH	pH	°C.	°C.	°C.	ohms	ohms	ohms	p p m.
Clay adobe No. 1 . . . . .	8.7	9.1		210	465					
Clay adobe No. 2 . . . . .	7.0		8.1	126		342	780	142	140	329
Silty clay loam No. 3 . . . . .	7.2	7.8	8.7	060	089	133	990	53	100	202
Fine sandy loam No. 4 . . . . .	7.0	9.3	9.5	077	108	221	705	53	100	193
Fine sandy loam No. 5 . . . . .	7.0		8.8	031		157	1055			189
Beach sand No. 6 . . . . .	6.5	8.4	9.9	035	2.25	369	560	53	100	868
Silica sand No. 7 . . . . .	6.2	9.7	10.0	0					60	76

TABLE 5  
EFFECTS OF ALKALI WATER "D" ON VARIOUS SOILS

Analyses of 1:5 Water Extracts																	
P P M Air Dry Soil																	
Description of Soils	Ph Soil Suspension			Freezing point depression at optimum moisture content			Specific Resistance (25°C) 1:1 Water Extract			Total Solids		Bicarbonate HCO <sub>3</sub>		Chloride Cl		Sulfate SO <sub>4</sub>	
	Un-treated soil	7 liters water per 9 kilos soil	14 liters water per 9 kilos soil	Un-treated soil	7 liters water per 9 kilos soil	14 liters water per 9 kilos soil	Un-treated soil	7 liters water per 9 kilos soil	14 liters water per 9 kilos soil	Un-treated soil	7 liters water per 9 kilos soil	14 liters water per 9 kilos soil	Un-treated soil	7 liters water per 9 kilos soil	14 liters water per 9 kilos soil	Un-treated soil	7 liters water per 9 kilos soil
Clay adobe No. 1 . . . . .	Ph	Ph	Ph	°C. .210	°C. .267	°C. .	ohms	ohms	ohms	p.p.m. 1200	p.p.m.	p.p.m. 281	p.p.m. 106	p.p.m. . . .	p.p.m. 327	p.p.m. . . . . .	p.p.m.
Clay adobe No. 2	7.0		7.1	126	.114		2025		1335	. . . .	970		98	. .	30	. . .	230
Silty clay loam No. 3	7.2	7.2	7.1	060	.077	058	1885	2465	745	545	73	61	18	10	93	117	
Fine sandy loam No. 4	7.0	6.7	7.8	.077	109		1060	1630	1070	785	61	73	18	20	150	113	
Fine sandy loam No. 5	7.0		7.7	031	.054		4970	2025	475	540	134	146	10	10	164	117	
Beach sand No. 6 . . .	6.5	5.3	7.3	035	.114	.127	780	1800	750	1255	acid	37	18	10	348	755	
Silica sand No. 7 . .	6.2	7.7	8.6	0						430	. . .	122	. . . .	10	. . .	105	

TABLE 6  
EFFECTS OF ALKALI WATER "E" ON VARIOUS SOILS

Description of Soils	Analyses of 1:5 Water Extracts											
	P. P. M. Air Dry Soil											
	Total Solids			Bicarbonate HCO <sub>3</sub>			Chloride Cl			Sulfate SO <sub>4</sub>		
	Specific Resistance (25°C.) 1:1 Water Extract			Freezing point depression at optimum moisture content			pH Soil Suspension					
	7 liters water per 9 kilos soil	14 liters water per 9 kilos soil	Un- treated soil	°C.	°C.	°C.	Un- treated soil	7 liters water per 9 kilos soil	14 liters water per 9 kilos soil	7 liters water per 9 kilos soil	14 liters water per 9 kilos soil	7 liters water per 9 kilos soil
Clay adobe No. 1.....	8.7	9.0	7.0	.210	.282	.156	.060	.073	.072	.077	.031	.035
Clay adobe No. 2.....	7.0	7.6	7.0	.126	.126	.156	.060	.073	.072	.077	.031	.035
Silty clay loam No. 3.	7.2	8.0	7.2	.060	.073	.067	.060	.073	.072	.077	.031	.035
Fine sandy loam No. 4.	7.0	8.1	7.0	.077	.072	.155	.077	.072	.072	.077	.031	.035
Fine sandy loam No. 5.	7.0	8.3	7.0	.031	.101	.101	.031	.101	.101	.101	.101	.101
Beach sand No. 6.....	6.5	7.8	6.5	.035	.189	.246	.035	.189	.246	.189	.246	.189
Silica sand No. 7.....	6.2	9.1	6.2	.035	.189	.246	.035	.189	.246	.189	.246	.189

TABLE 7  
EFFECTS OF ALKALI WATER "F" ON VARIOUS SOILS

Analyses of 1:5 Water Extracts																
P. P. M. Air Dry Soil																
Description of Soils	pH			Freezing point depression at optimum moisture content			Specific Resistance (25°C) 1:1 Water Extract			Total Solids		Bicarbonate HCO <sub>3</sub>	Chloride Cl	Sulfate SO <sub>4</sub>		
	Un-treated soil	7 liters water per 9 kilos soil	14 liters water per 9 kilos soil	Un-treated soil	7 liters water per 9 kilos soil	14 liters water per 9 kilos soil	Un-treated soil	7 liters water per 9 kilos soil	14 liters water per 9 kilos soil	7 liters water per 9 kilos soil	14 liters water per 9 kilos soil	7 liters water per 9 kilos soil	14 liters water per 9 kilos soil	7 liters water per 9 kilos soil	14 liters water per 9 kilos soil	
Clay adobe No. 1	pH 8.7	pH 8.6	pH	°C. 210	°C. 516	°C.	ohms	ohms	ohms	p. p. m. 1575	p. p. m.	p. p. m. 281	p. p. m.	p. p. m. 426	p. p. m.	p. p. m. 257
Clay adobe No. 2	7.0		6.7	.126		.861	2025		275		3205		85		1130	202
Silty clay loam No. 3	7.2	7.0	6.7	.060	120	433		1145	330	765	2670	73	49	195	1050	64
Fine sandy loam No. 4	7.0	7.4	7.2	.077	.220	633	1655	705	310	1155	2840	98	61	195	980	741
Fine sandy loam No. 5	7.0	...	7.3	.031		433	4970	310	310	1290	2265	98	122	460	910	78
Beach sand No. 6	6.5	4.5	6.5	.035	299	.643	4230	760	480	1650	1950	acid	37	195	730	852
Slice sand No. 7	6.2	6.8	6.7	0							1735		39		530	78

with the characteristic of high primary alkalinity, it is evident that the effect on the OH ion concentration of the soil varies significantly, depending on the soil. Soil 1, a clay adobe, is already very alkaline and no appreciable changes occur in the OH ion concentration. Soil 2, another clay adobe, of practically neutral reaction, is increased only slightly in OH ion concentration, even by the larger proportions of water. In soil 3, a silty clay loam, the effect on the alkalinity is more marked, but becomes excessive only in the case of water C, which has a high percentage of primary alkalinity as well as a high concentration value. The fine sandy loams, soils 4 and 5, are influenced very decidedly in their alkalinity even by a water of such low concentration as E. Some of the OH ion concentrations found in these sandy soils are very high from the standpoint of plant nutrition. In the beach sand and pure silica sand the maximum effects are observed. Clearly we may draw the general conclusion from the above data that, other conditions being equal, a far greater effect is produced on the alkalinity of a sandy type of soil than on that of a heavy soil.

It may reasonably be assumed that the explanation of these observations rests on the different chemical and physical composition of the various soils. Possibly in the soils containing large clay fractions chemical reactions take place between some of the silicates and the added alkaline salts, resulting either in precipitation or the formation of new silicate compounds. Surface adsorption also doubtless plays some part in the reduction of alkalinity noted. In the sandy soils these chemical and physical reactions are much less marked and the original alkalinity of the irrigation water is therefore more effective in increasing the alkalinity of the soil solution. In the pure sand the alkalinity is simply the resultant of the chemical reactions taking place between the constituents of the water as evaporation proceeds, the final OH ion concentration being dependent on the state of equilibrium existing between  $\text{CO}_2$ ,  $\text{HCO}_3^-$ ,  $\text{CO}_3^{2-}$ , etc.

We may next inquire how the measurements obtained by the use of the hydrogen electrode compare with results derived from titrating water extracts for  $\text{CO}_2$  and  $\text{HCO}_3^-$ , with phenolphthalein and methyl orange as indicators. In only two cases are normal carbonates found in the soil extracts, even when the OH ion concentration of the soil is high. In the soils with the highest concentration of this ion are also found high values for the  $\text{HCO}_3^-$  titration, but there is no exact relationship. A very significant difference may be found in OH ion concentration without a corresponding difference in titrations. An

agreement could scarcely be expected, inasmuch as the OH ion concentration is the resultant of numerous factors which influence the dissociation and hydrolysis of the carbonates and silicates present, while the titration represents a total content of soluble alkaline salts capable of producing OH ion as the equilibrium changes during the titration. These considerations would suggest that the determination of the OH ion concentration of the soil has greater value than the customary titration. By using the colorimetric method suggested by Gillespie<sup>3</sup> in place of the hydrogen electrode the OH ion determination could be made more conveniently, perhaps, than the titration. It should also be considered that during the filtration through the Pasteur filter under added pressure of air (and CO<sub>2</sub>) the relation of CO<sub>3</sub> and HCO<sub>3</sub> may be changed.

The total concentration of the soil solution as evidenced by freezing point determinations is particularly affected by the waters having a high percentage of primary salinity. In this case also the nature of the soil is of great importance. The greatest effect is produced in the soils of lighter texture. The exact concentration, of course, is dependent upon the moisture content of the soil, for, as Bouyoucos<sup>4</sup> has shown, in heavy soils only a small proportion of the total water is present in the free state. With similar decreases in moisture content, therefore, a much greater increase in concentration of solution may take place in a heavy soil than in a sandy one. The total solids in the extracts reflect fairly well the relative concentrations in the soil solution, although the freezing point depressions may frequently give a better picture of the probable injurious effects on plants, since it is the concentration of the soil solution and not that of the extract which is important. The total concentration of the extract may be estimated very conveniently by the conductivity method.

In table 8 are presented data for a number of California soils commonly recognized as "alkali" soils. Comparisons may be made between these and the normal soils treated with the alkali waters. It will be noted that in a number of cases the comparatively limited amounts of water used in the experiment have brought about OH ion concentrations comparable with those found in the so-called "black alkali" soils. The total concentration of salts is extremely high in a number of these soils and several times the quantity of the most concentrated water would have been necessary to reproduce this condition in the soils under investigation. These higher concentrations, however, are extremely detrimental or even fatal to plant growth.



TABLE 8  
CHARACTERISTICS OF VARIOUS ALKALI SOILS

No.	pH	Freezing point depression* °C.	Specific Resistance (25°C.) 1:1 Water Extract	Analyses of 1:5 Water Extracts P.P.M. Air Dry Soil					Remarks
				Total Solids	Carbonate CO <sub>3</sub>	Bi-carbonate HCO <sub>3</sub>	Chlorine Cl	Sulfate SO <sub>4</sub>	
			ohms	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	
1	9.4	.869	90	9650	120	415	1684	2251	"white alkali", surface 6 inches, 20% germination.
2	8.7	1.044	205	4150	none	134	1117	1239	"white alkali," surface 6 inches, stunted seedlings.
3	8.3	.786	140	10790	none	134	3511	2708	Marshy spot, stunted seedlings.
5	8.0	4.600	45	30260	none	98	6418	12296	No germination.
8	9.2	2.400	55	17850	132	317	3617	6313	"white alkali," stunted seedlings.
9	10.4	1.590	115	7000	1692	659	479	1144	"black alkali," no germination.
10	9.9	.872	205	3935	348	256	745	897	20% germination.

\*Freezing point determinations made at optimum moisture content.

It is now desirable to discuss the foregoing data from a general point of view and with reference to certain previous publications. Much of the earlier work in this field was due to Hilgard,<sup>5</sup> and many of the facts and ideas presented by him are still in use as a basis for judging the value of waters for irrigation purposes. It seems very necessary to modify these first teachings, however, in the light of more recent experiments. It is highly essential that the soil be considered as a chemical system in which the effect of any water will be modified by the chemical and physical reactions in the soil. The views of Kelley<sup>6</sup> in this connection have already been referred to, and some experiments by Lipman and Gericke<sup>7</sup> also suggest the necessity of considering the effect of the soil. These investigators found, by examining water extracts of a soil to which various alkali salts had been added, that less of  $\text{Na}_2\text{CO}_3$  was recovered than of  $\text{NaCl}$  or  $\text{Na}_2\text{SO}_4$ . Evidently it is not sufficient to calculate simply the quantity of sodium salts added to an acre of soil by a given number of inches of irrigation water. Such a method of calculation does not take into account the chemical and physical reactions in the soil nor the precipitations occurring when the water evaporates.

The general statement is often made that an alkali water may be used without danger if the soil is of a well drained sandy type, but may become injurious when applied to a heavy soil. In view of data such as those presented in this paper it should be recognized that the possibility of injury may be much greater in a sandy soil than in a heavy soil unless the sandy soil is completely drained. If this drainage is prevented by any underlying impervious strata it would seem possible that a condition distinctly injurious to many plants might be quickly produced in a sandy soil. This seems to be particularly true of waters tending to increase the OH ion concentration of the soil solution. That a relatively slight increase in OH ion concentration may be unfavorable to seedlings has been shown in a previous investigation.<sup>8</sup>

The above conclusions have received confirmation in a recent article by Harris and Pittman.<sup>4</sup> In an extensive investigation in which freezing point determinations were made as well as analyses of water extracts it was shown that the soil exerts a most pronounced effect on the salts added, particularly in the case of  $\text{Na}_2\text{CO}_3$ . In connection with calculations based on freezing point depressions made by the authors just cited, it may be useful to point out that it is not possible to compute the extent to which a given quantity of salt will increase

the depression of the freezing point in a soil of optimum moisture content, unless account is taken of the free and unfree water as proposed by Bouyoucos.<sup>1</sup> Thus if the total moisture content is used in the calculations it might appear that the depression was greater than could be accounted for by the added salt, since the water effective for solution is less than the total water present.

The data on which a correlation of injury to the plant and the composition and concentration of the soil solution could be based are very inadequate. Certain previous experiments<sup>a</sup> have shown that the environment for seedlings may become unfavorable with comparatively slight increases in OH ion concentration. High total concentrations of any salts are unfavorable, but the exact relations between the ions which may bring about malnutrition of the plant remain to be worked out. In order to gain a preliminary idea of the effect of the total and of the OH ion concentrations brought about in the various soils after treatment with the waters described in this paper, portions of the treated soils were placed in tumblers and germination tests were made with barley and pea seeds. The inhibiting effect of high OH ion concentrations was especially noted in this experiment. In certain cases still other factors might enter into the question; for example, there is a possibility that with higher concentrations of OH ion the solubility of Fe or  $\text{PO}_4$  might be decreased, perhaps bringing about such low concentrations of these elements in the soil solution that the plant would be unable to obtain sufficient quantities for its nutrition. In brief, the whole field is so complicated that it appears advisable to investigate one by one such factors as admit of definite measurement. It is hoped that this paper may suggest certain possibilities in this direction.

## SUMMARY

1. A number of different waters of interest from the standpoint of irrigation have been applied to soils of several types. The effects on the freezing point depressions and on the OH ion concentrations of the soils have been determined.

2. Waters with a high percentage of primary alkalinity applied even in moderate quantities produced greatly increased OH ion concentrations. The effect was far greater in sandy soils than in heavy soils.

3. Waters with a high concentration of alkali salts soon produced excessive concentrations in the soil solution as shown by the freezing point depressions. This was especially true of sandy soils.

4. The determination of the OH ion concentration and freezing point depression of soils frequently may be more convenient in practice and more useful as a basis of interpretation than the ordinary procedures used in analyzing alkali soils.

5. Any appreciable increases in OH ion concentration of the soil (especially when exceeding PH 8.5) appear to be injurious to seedlings.

6. It is suggested that the geological method of classifying waters and the use of reaction values provide methods of expression which are very useful in interpreting the results of analyses of waters intended for irrigation purposes.

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**A NEW AND SIMPLIFIED METHOD FOR THE  
STATISTICAL INTERPRETATION OF  
BIOMETRICAL DATA**

BY  
GEORGE A. LINHART

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A NEW AND SIMPLIFIED METHOD FOR THE  
STATISTICAL INTERPRETATION OF  
BIOMETRICAL DATA<sup>1</sup>

BY  
GEORGE A. LINHART

SECTION I

The derivation of the Law of Probability may be found in any text on the subject. Here we shall assume its validity and use it to obtain the several quantities which serve as criteria in statistical calculations.

In the fundamental equation

$$y = ke^{-h^2x^2} \quad [1]$$

there are two characteristic constants,  $k$  and  $h$ , whose numerical values must be known for a given set of data before we can proceed with any calculations. A simple and at the same time exact method of obtaining the numerical values for those constants forms the subject of this paper.

Since  $y$  equals  $k$  when  $x$  equals zero,  $k$  is the probability of an error zero and will therefore be defined here as the largest number of measurements of a given set having the same numerical value; while  $y$  will denote any number of measurements whose group value ranges from zero to the group value of the number of measurements denoted by  $k$  or  $y_0$ . Equation (1) then becomes

$$\frac{y}{y_0} = e^{-h^2x^2} \quad [2]$$

which by means of logarithms we have transformed into a linear equation,

$$\text{Log } (2.303 \text{ Log } \frac{y_0}{y}) = 2 \text{ Log } x + 2 \text{ Log } h \quad [3]$$

or

$$\text{Log } (\text{Log } \frac{y_0}{y}) = 2 \text{ Log } x + 2 \text{ Log } h - 0.3623 \quad [4]$$

Collecting  $2 \text{ Log } h$  and  $-0.3623$  into one constant, we have,

$$\text{Log } (\text{Log } \frac{y_0}{y}) = 2 \text{ Log } x + K \quad [5]$$

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<sup>1</sup>From the Division of Soil Chemistry and Bacteriology, College of Agriculture, University of California, Berkeley.

If we now plot  $\text{Log} (\text{Log } y_0 - \text{Log } y)$  as ordinate and  $\text{Log } x$  as abscissa with a slope of 2 all the measurements should theoretically fall on the straight line, provided the data are susceptible to statistical interpretation—that is, provided they are truly *chance* data. Practically, however, even such data fall on either side of the straight line. Drawing now the “best” straight line with a slope of 2 through these points, we can then read off the values on the line as accurately as we choose, depending upon the size of the scale of plotting, and construct a “theoretical” frequency curve for comparison with the experimental frequency curve obtained in the usual way; that is, by plotting the number of experiments in groups or classes against the measured values. Frequently  $y_0$  does not fall directly over the arithmetical mean. In such a case the theoretical polygon may be shifted to the left or to the right, and this corresponds to the parallel shifting of the straight line from which the values for the construction of the theoretical frequency polygon have been obtained. Often this theoretical polygon reveals the fact that the arithmetical mean calculated from the “raw” data is not in all cases the “best” mean, for, as it frequently happens, one or two abnormal values will vitiate the mean considerably, especially if the number of experiments are not sufficiently large. We must, therefore, so superpose the two polygons as to make their areas approximately equivalent, since, as will be shown later, the areas play an important part in the calculation of the probable error. A concrete example will best illustrate the method of procedure.

In a recent paper by Waynick and Sharp (1919) are given the nitrogen contents of a hundred samples of a local soil. The results are recorded to 0.001%, based upon ten gram samples, and therefore to 0.1 mg. In figure I these one hundred results are mapped in groups or classes 0.1 mg. apart, the circles indicating the number of determinations falling into each class.<sup>2</sup> Plotting these classes vertically to a scale of one-half inch per one determination, we obtain the multimodal curve drawn immediately above the circles. Evidently the analyses are too fine as compared with the variability of nitrogen in those samples of soil. The number of determinations were then grouped in classes 0.5 mg. apart, resulting in the next curve above. This curve bears some resemblance to a “frequency” curve, but is still unsatisfactory. However, such a curve is quite sufficient for the construction of a theoretical frequency polygon by our straight line method. In this case  $y_0$  would equal 25 and could be made to fall directly over the arithmetical mean, 10.0 mg.

<sup>2</sup>When the circles fall on a line dividing two classes, then if the number of circles is even they are equally divided between the two classes; if odd, the extra one is put into that class which helps to make the experimental polygon most symmetrical.

Indeed, if we attempt to plot these data in classes very much farther apart than 0.5 mg., say 2.0 mg., we obtain a so-called skew curve, and, finally, we may obtain a line sloping in one direction only when we plot these data in classes 2.5 mg. apart. It is evident, therefore, that such skew curves are meaningless.<sup>3</sup> In the present case when we plot the data in classes 1.0 mg. apart the curve "skews" but slightly. Here  $y_0$  falls directly over the arithmetical mean, and the one hundred determinations fall into four classes. With these four points on the curve, two on each side of the mean and approximately equidistant from it we may construct the straight line as shown in figures VII, VIII and IX, where the values for  $\text{Log} (\text{Log } y_0 - \text{Log } y)$  are plotted as ordinates and the values for  $\text{Log } x$  as abscissae,  $x$  denoting the residuals on either side of the mean without regard to algebraic sign. It should be noted that in drawing the "best" straight line with the theoretical slope of 2 through such points proportionately less weight must be given to points taken from the experimental polygon near the base than to those taken from the upper portion of the curve. A little practice will soon enable one to judge at a glance which points are most significant. Having now obtained the "best" straight line, we may calculate any number of values for  $x$  by means of equation (5), namely:

$$\text{Log} (\text{Log } \frac{y_0}{y}) = 2 \text{Log } x + K,$$

$K$  denoting the distance on the  $\text{Log} (\text{Log } y_0 - \text{Log } y)$  axis, or ordinate, from the origin to the point of its intersection by the "best" straight line. In the present example  $y_0$  equals 40 and  $y$  may be taken anywhere from one to thirty-nine, but for the construction of the theoretical polygon six to ten values for  $y$  will suffice. These are shown in table I.

#### DISCUSSION OF THE FIGURES

Figure I has been fully discussed. Figure II is but another example of how to construct a theoretical polygon approximately equivalent in area to the experimental polygon. An interesting set of data is that mapped in figure III. Here the total nitrogen in each sample is so small that a few samples might have contained no measurable amount of nitrogen at all. The values for the construction of these two figures, II and III, were taken from a paper by Waynick (1918).

The data mapped in figure IV are recorded in a paper by Batchelor and Reed (1918). Here as in figure III the theoretical polygon indicates that among the one thousand orange trees about three might have borne

<sup>3</sup>A discussion of truly abnormal curves and their susceptibility to statistical interpretation will be given in another paper. See also section II of this paper.

no fruit at all had they been left wholly to chance. In fact one tree yielded but five pounds of fruit, which is practically zero, while another yielded 341 pounds, the mean of all the thousand trees being 137.6 pounds of fruit. Two more interesting sets of data are those of Wood (1910) on the dry weights of mangel roots, and by Collins (1912) on butter fat. These results are mapped in figures V and VI. In figures VII, VIII and IX are shown the construction of the straight lines from the experimental data as previously described. Finally, in figure X are mapped the results of bacterial counts taken from a recent article in *Science* (1920).

#### CALCULATION OF THE INDEX OF PRECISION

Turning once more to the straight line plots on figures VII, VIII and IX, we see that we may read off the values for  $K$  of equation (5) to any degree of accuracy, depending upon the size of the scale of the plot. On the above plots, 20x20 inches, the values for  $K$  can be read off accurately to three places of decimals, which is quite sufficient for most cases. With this value for  $K$  of a given set of measurements we can calculate the value for  $h$ , the Index of Precision, as is shown in equation (5) where  $K$  was put in place of  $2 \log h - 0.3623$ ; hence,

$$h = (10)^{\frac{K + 0.3623}{2}} \quad [6]$$

#### CALCULATION OF THE PROBABLE ERROR

The simplest way of calculating the probable error is to take from a probability integral table the value for  $hx$  corresponding to the integral value  $\frac{1}{2}$ . This value for  $hx$  is 0.4769; hence,

$$x = 0.4769(10)^{-\frac{K + 0.3623}{2}} \quad [7]$$

We might of course draw a straight line through every "class" point parallel to the "best" straight line and so obtain a probable error for each class which, when meaned, would give an average probable error. However, in most cases the probable error obtained from the "best" straight line is more accurate.

A more instructive method of calculating the probable error is to make a tracing of the theoretical polygon, which is constructed from the values read off on the straight line plot, on reasonably uniform tracing cloth and then carefully cutting out the area under this curve, rolling it up and finally weighing it on accurate balances. The polygon is then unrolled and folded along the mode exactly in two and trimmed along the sides parallel to the fold by means of a photographer's print trimmer

until it weighs exactly one-half of the original weight. Replacing now this trimmed tracing upon the original theoretical polygon, we may read off the probable error on the base of the polygon at the limit of the tracing.

#### CALCULATION OF THE PROBABLE ERROR OF THE ARITHMETICAL MEAN

By means of the Principle of Least Squares it can be shown that the probable error of the arithmetical mean,  $x_0$ , is equal to the probable error (obtained from  $h$ ) of one determination divided by the square root of the number of determinations, or,

$$x_0 = \frac{x}{\sqrt{n}} = \frac{0.4769}{\sqrt{n}} (10) \frac{K + 0.3623}{2}$$

#### TABLES OF RESULTS

In the tables below are given in the first columns the number of determinations falling into each class, while in the last columns are given the values calculated by means of the straight lines for the construction of the theoretical polygons. The headings are self-explanatory. The Roman numerals of each table correspond to the Roman numerals on the figures constructed from these tables.

I						
						Calculated from $K = -0.670$
$y$	$\text{Log } \frac{y_0}{y}$	$\text{Log (Log } \frac{y_0}{y})$	$x$ obs.	$\text{Log } x$	$\text{Log } x$	$x$
0	$+\infty$	$+\infty$			$+\infty$	$\pm \infty$
1	1.602	+0.205	.....		+0.4375	2.739
3	1.125	+0.051	2.0	+0.301	+0.3605	2.293
5	0.903	-0.044	.....		+0.3130	2.056
9	0.648	-0.188	2.0	+0.301	+0.2410	1.742
15	0.426	-0.371	.....		+0.1495	1.411
20	0.301	-0.521			+0.0745	1.187
22	0.260	-0.586	1.0	0.000	+0.0420	1.102
26	0.187	-0.728	1.0	0.000	-0.0290	0.935
30	0.125	-0.903	.....		-0.1165	0.765
35	0.058	-1.237	.....		-0.2835	0.192
40	0.000	$-\infty$	.....		$-\infty$	0.000

II						
						Calculated from $K = -0.350$
$y$	$\text{Log } \frac{y_0}{y}$	$\text{Log (Log } \frac{y_0}{y})$	$x$ obs.	$\text{Log } x$	$\text{Log } x$	$x$
0	$+\infty$	$+\infty$	.....		$+\infty$	$\pm \infty$
1	1.342	+0.128	1.7	+0.230	+0.239	1.734
2	1.041	+0.018	.....		+0.184	1.528
3	0.865	-0.063	1.8	+0.255	+0.144	1.392
6	0.564	-0.249	.....		+0.051	1.124
8	0.439	-0.357	.....		-0.004	0.991
10	0.342	-0.466	0.8	-0.097	-0.058	0.875
15	0.166	-0.780	0.7	-0.155	-0.215	0.610
19	0.064	-1.194	0.2, or 0.3	-0.699, or -0.523	-0.422	0.378
22	0.000	$-\infty$	.....	.....	$-\infty$	0.000

## III

Calculated from  
 $K = +0.250$ 

$y$	$\text{Log } \frac{y_0}{y}$	$\text{Log } (\text{Log } \frac{y_0}{y})$	$x$ obs.	$\text{Log } x$	$\text{Log } x$	$x$
0	$+\infty$	$+\infty$	.....	.....	$+\infty$	$\pm \infty$
1	1.447	+0.161	1.15	+0.061	-0.045	0.902
2	1.146	+0.057	.....	.....	-0.097	0.801
4	0.845	-0.073	0.85	-0.071	-0.162	0.690
7	0.602	-0.220	.....	.....	-0.235	0.582
8	0.544	-0.264	0.55	-0.260	-0.257	0.553
10	0.447	-0.350	.....	.....	-0.300	0.510
15	0.271	-0.567	.....	.....	-0.409	0.390
18	0.192	-0.717	0.35	-0.456	-0.484	0.328
21	0.125	-0.903	0.25	-0.602	-0.577	0.265
25	0.049	-1.310	.....	.....	-0.779	0.166
28	0.000	$-\infty$	.....	.....	$-\infty$	0.000

## IIIa

Observed

Calculated from  $K = 5.625$ 

$y$	$\text{Log } \frac{y_0}{y}$	$(\text{Log } \frac{m}{m_0})^2$	$\text{Log } m$	$(\text{Log } \frac{m}{m_0})^2$	$\text{Log } \frac{m}{m_0}$	$\text{Log } m$
0	$+\infty$			$+\infty$	$\pm \infty$	$\pm \infty$
1	1.591			0.2828	0.5318	+0.3318 -0.7318
5	0.892	0.2304	+0.28 -0.68	0.1585	0.3981	+0.1981 -0.5981
10	0.591			0.1051	0.3241	+0.1241 -0.5241
18	0.336	0.0576	+0.04 -0.44	0.0597	0.2443	+0.0443 -0.4443
19	0.312	0.0576	+0.04 -0.44	0.0555	0.2356	+0.0356 -0.4356
30	0.114			0.0203	0.1425	-0.0575 -0.3425
35	0.047			0.0084	0.0917	-0.1083 -0.2917
39	0.000			0.0000	0.0000	-0.2000

## IV

Calculated from  
 $K = -4.100$ 

$y$	$\text{Log } \frac{y_0}{y}$	$\text{Log } (\text{Log } \frac{y_0}{y})$	$x$ obs.	$\text{Log } x$	$\text{Log } x$	$x$
0	$+\infty$	$+\infty$	.....	.....	$+\infty$	$\pm \infty$
1	2.182	+0.339	137.6, or 202.4	+2.139, or 2.306	+2.220	165.8
2	1.881	+0.274	102.4	2.211	2.187	153.8
3	1.705	+0.232	182.4	2.261	2.166	146.6
7	1.337	+0.126	142.4	2.154	2.113	129.7
8	1.279	+0.107	117.6	2.070	2.103	126.9
17	0.952	-0.021	122.4	2.088	2.039	109.5
20	0.881	-0.055	102.4	2.010	2.022	105.3
25	0.784	-0.106	97.6	1.989	1.997	99.3
51	0.474	-0.324	82.4	1.916	1.886	76.9
58	0.419	-0.378	77.6	1.890	1.861	72.6
62	0.390	-0.409	62.4	1.795	1.846	70.1
91	0.223	-0.652	42.4	1.627	1.724	53.0
116	0.118	-0.928	57.6	1.760	1.585	38.5
120	0.103	-0.987	37.6	1.575	1.556	36.0
124	0.089	-1.051	17.6	1.246	1.525	33.5
142	0.030	-1.523	22.4	1.350	1.288	19.4
152	0.000	$-\infty$	.....	.....	$-\infty$	0.0

Calculated from $K = -0.980$						
$y$	$\text{Log } \frac{y_0}{y}$	$\text{Log (Log } \frac{y_0}{y})$	$x$ obs.	$\text{Log } x$	$\text{Log } x$	$x$
0	$+\infty$	$+\infty$			$+\infty$	$\pm\infty$
1	1.623	+0.210	4.0, or 5.0	+0.602, or +0.699	+0.595	3.94
2	1.322	+0.121	4.0	+0.602	+0.551	3.55
7	0.778	-0.109	3.0	+0.477	+0.436	2.73
9	0.669	-0.175	3.0	+0.477	+0.403	2.53
16	0.419	-0.378	2.0	+0.301	+0.301	2.00
17	0.393	-0.406	2.0	+0.301	+0.287	1.94
24	0.243	-0.614			+0.183	1.52
32	0.118	-0.928	1.0	0.000	+0.026	1.06
33	0.104	-0.983	1.0	0.000	+0.002	1.00
38	0.043	-1.367			-0.194	0.64
42	0.000	$-\infty$			$-\infty$	0.00

## VI

Calculated from $K = -0.700$						
$y$	$\text{Log } \frac{y_0}{y}$	$\text{Log (Log } \frac{y_0}{y})$	$x$ obs.	$\text{Log } x$	$\text{Log } x$	$x$
0	$+\infty$	$+\infty$			$+\infty$	$\pm\infty$
1	2.415	+0.383	0.85	-0.071	-0.159	0.694
3	1.938	+0.287	0.95	-0.022		
4	1.813	+0.258	0.65, or 0.75	-0.187, or -0.125		
5	1.716	+0.235	0.85	-0.071		
7	1.570	+0.196	0.75	-0.125		
8	1.512	+0.180	0.65	-0.187		
11	1.374	+0.138	0.55	-0.260	-0.281	0.524
34	0.884	-0.054	0.45	-0.347		
39	0.824	-0.084	0.55	-0.260	-0.392	0.406
45	0.762	-0.118	0.45	-0.347		
58	0.652	-0.186	0.35	-0.456		
63	0.616	-0.210	0.35	-0.456	-0.455	0.351
97	0.428	-0.369	0.25	-0.602		
137	0.278	-0.556	0.25	-0.602	-0.628	0.236
200	0.114	-0.943	0.15	-0.824	-0.822	0.151
205	0.103	-0.987	0.15	-0.824		
241	0.033	-1.481	0.05	-1.301	-1.091	0.081
260	0.000	$-\infty$			$-\infty$	0.000

## X

Calculated from $K = -2.690$						
$y$	$\text{Log } \frac{y_0}{y}$	$\text{Log (Log } \frac{y_0}{y})$	$x$ obs.	$\text{Log } x$	$\text{Log } x$	$x$
0	$+\infty$	$+\infty$			$+\infty$	$\pm\infty$
1	0.903	-0.044	20, or 30	+1.301, or +1.477	+1.323	21.
3	0.426	-0.371			+1.160	14.
5	0.204	-0.690	10.	+1.000	+1.000	10.
7	0.058	-1.237			+0.727	5.
8	0.000	$-\infty$			$-\infty$	0.

<i>y</i>	Observed		<i>X<sub>a</sub></i>	Calculated from <i>K</i> = 5.625		
	Log $\frac{y_0}{y}$	(Log $\frac{m}{m_0}$ ) <sup>2</sup>	Log <i>m</i>	(Log $\frac{m}{m_0}$ ) <sup>2</sup>	Log $\frac{m}{m_0}$	Log <i>m</i>
0	+ ∞			+ ∞	± ∞	± ∞
0.2	1.477			0.6029	0.7765	+1.9765 +0.4235
0.5	1.079			0.4404	0.6636	+1.8636 +0.5364
1	0.778			0.3176	0.5636	+1.7636 +0.6364
2	0.477	0.160	+1.60 +0.80	0.1947	0.4413	+1.6413 +0.7613
5	0.079	0.040	+1.40 +1.00	0.0322	0.1794	+1.3794 +1.0206
6	0.000			0.0000	0.0000	+1.2000

SUMMARY TABLE

In the table following the Roman numerals in the first column refer either to the tables or to the figures themselves; in the second, third and fourth columns are given the means the probable errors and the probable errors of the means calculated by the new method. In the fifth, sixth and seventh columns are given the means, the probable errors and the probable errors of the means taken from the literature listed at the end of this article.

By the New Method				Taken from the Literature			
Tables	Mean	Probable error	Probable error of the mean	Mean	Probable error	Probable error of the mean	
I	10.0	0.68	0.068	10	0.60	0.060	W. & S.
II	2.7	0.47	0.052	2.7	0.47	0.052	W.
III	0.65	0.24	0.026	0.7	0.24	0.026	W.
IV	137.6	35.26	1.12	137.6	37.0	1.2	B. & R.
V	14.5	0.97	0.08	14.5	1.1	0.087	Wood
VI	3.05	0.140	0.004	3.07	0.158 <sub>0</sub>	0.004	Collins
X	15.0	7.0	2.0				Science

oThis value is erroneous. Apparently an arithmetical mistake.

SECTION II

One of the fundamental postulates of the law of probability of errors is that positive and negative errors are equally frequent. This however is not generally true. It is true for example in military statistics where the deviations from the arithmetic mean are small. Thus in measuring the heights of soldiers the maximum deviation from the mean is never more than about one foot, while the height of the shortest soldier is about five feet. But if we wish to ascertain say the average number of children per family in the United States the frequency curve shows that some families may have *negative* children. For if the average be four children per family, and we *know* that some families have as many as



five times that number, then, according to the above postulate the frequency curve must include the count zero and beyond. This is typical of a great many cases and is rather the rule than the exception.

Let us now once more examine some of the figures of the previous section of this paper. It is not only conceivable but very likely that some of the samples of soil of which the nitrogen contents are mapped in figure 3 might have yielded no measurable amount of nitrogen. But the frequency curve indicates that some of the samples might have contained a quantity less than zero. The same is true of the yield of oranges mapped on figure IV and of the bacterial counts mapped on figure X. It must not however be concluded from this that the law of probability of errors does not apply to these cases. It is the particular form of the mathematical expression for the law of probability of errors which does not apply. We have therefore sought an equation of such form that it should satisfy the postulates of the law of probability of errors and also agree with experience. This equation is

$$\frac{y}{y_0} = e^{-h^2 (\text{Log} \frac{m}{m_0})^2} \quad [9],$$

where  $m$  denotes the numerical value of any measurement and  $m_0$  the value of the *geometric mean*.\* The meanings of  $y$ ,  $y_0$  and  $h$  are the same as those of the same quantities in equation (2). Equation (9) states that it is as likely or rather as unlikely that some values for  $m$  be zero as  $+\infty$ ; that is, in either case  $y/y_0$  would equal zero. When  $m$  equals  $m_0$ ,  $y/y_0$  equals 1; that is, the maximum probability is attained when the measured values do not deviate from the value of the mean. Transforming equation (9) into a rectilinear one, as has been done with equation (2), we obtain

$$\text{Log} \frac{y_0}{y} = 2.303h^2 (\text{Log} \frac{m}{m_0})^2 \quad [10],$$

or

$$\text{Log} \frac{y_0}{y} = K (\text{Log} \frac{m}{m_0})^2 \quad [11].$$

Whence  $h$ , the index of precision, equals  $\sqrt{K/2.303}$ , [12].

We may now proceed with the construction of the experimental polygons with the values given in columns 1 and 4 of tables IIIa and Xa, then find the "best" values for  $K$  from the straight line equation (11) and finally construct the theoretical curves from the values given in columns 1 and 7 of tables IIIa and Xa. The curves so obtained are shown in figures IIIa and Xa. From these curves the probable errors may be calculated as described in section I.

\*For a mathematical discussion see Galton, and McAlister, Proc. Roy. Soc. Lond. 29:365 (1879).

## SUMMARY

In section I of this paper, the usual mathematical expression for the law of the probability of errors has been transformed into a rectilinear form. With the aid of this equation, the statistical criteria for various sets of data may be very accurately calculated without previously finding, squaring, and so on, of the individual residuals, and thus may be saved an enormous amount of time and labor.

In section II, it is shown that the mathematical expression for the law of the probability of errors generally used holds only where the percentage deviations from the mean are small. A general equation is then developed, of which the former is but a special case. For when the percentage deviations from the mean are small, that is, when  $m$  is less than  $2 m_0$ , where  $m$  denotes the value of any measurement and  $m_0$  the value of the mean, our general equation

$$\frac{Y}{Y_0} = e^{-h^2 (\text{Log} \frac{m}{m_0})^2}$$

may be expanded in series, thus:

$$\text{Log} \frac{Y_0}{Y} = h^2 \left[ \left( \frac{m}{m_0} - 1 \right) - 1/2 \left( \frac{m}{m_0} - 1 \right)^2 + 1/3 \left( \frac{m}{m_0} - 1 \right)^3 - \dots \right]^2$$

Neglecting all terms but the first on the right-hand side, we obtain,

$$\text{Log} \frac{Y_0}{Y} = h^2 \left( \frac{m - m_0}{m_0} \right)^2$$

which is identical with the ordinary law of the probability of errors generally used, and most often misused, for, as has been pointed out, this equation holds only where the percentage deviations from the mean are small.

*Transmitted April 29, 1920. .*

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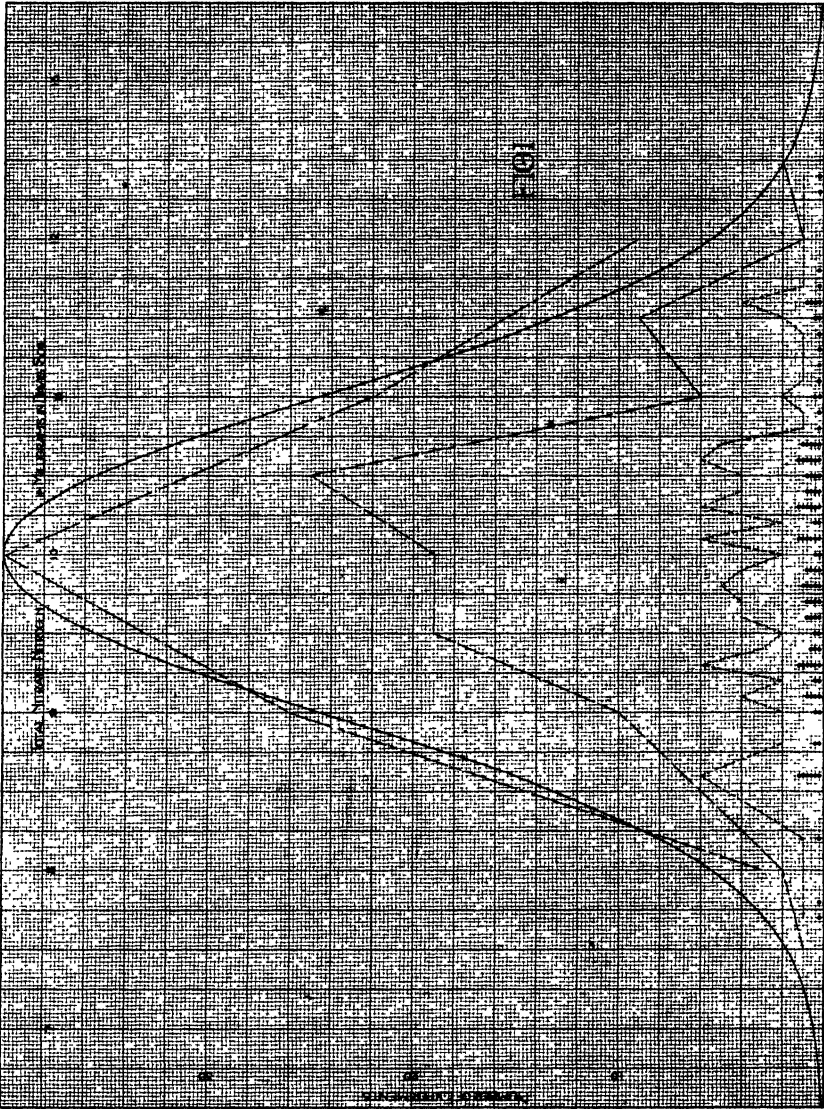
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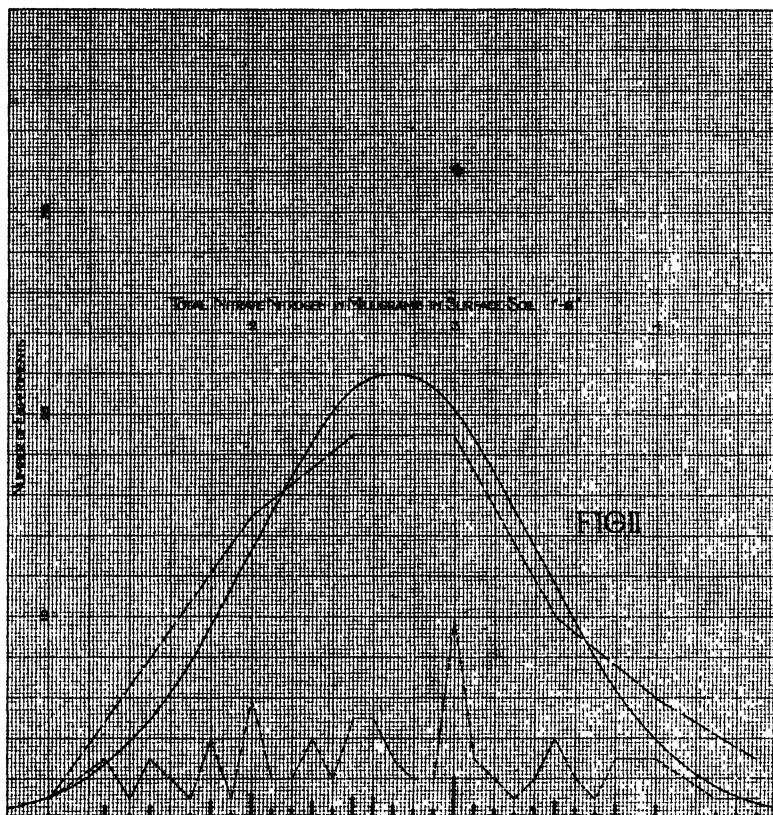
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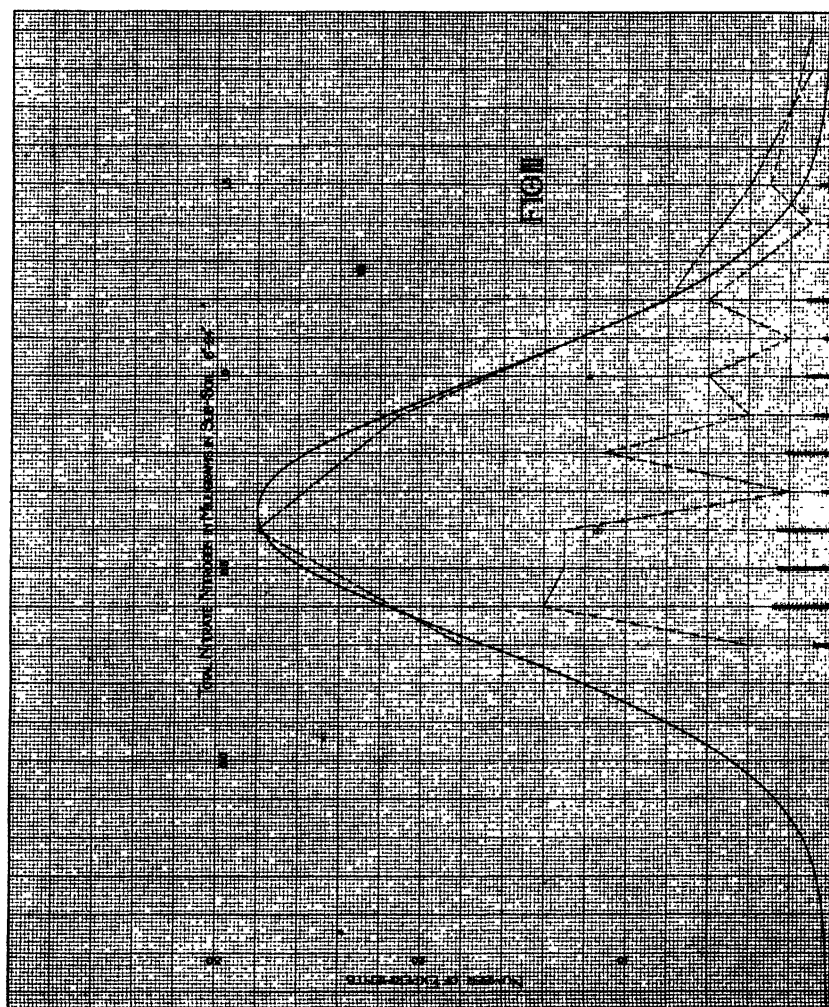
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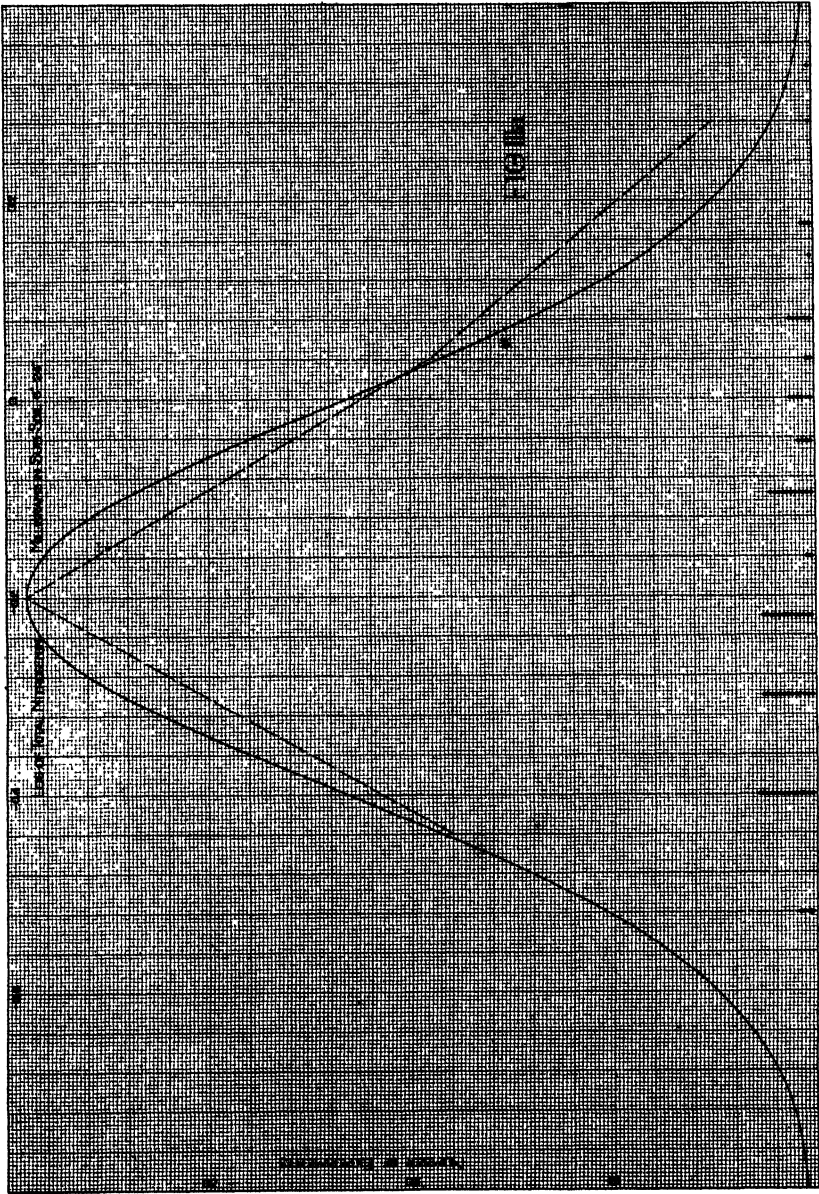
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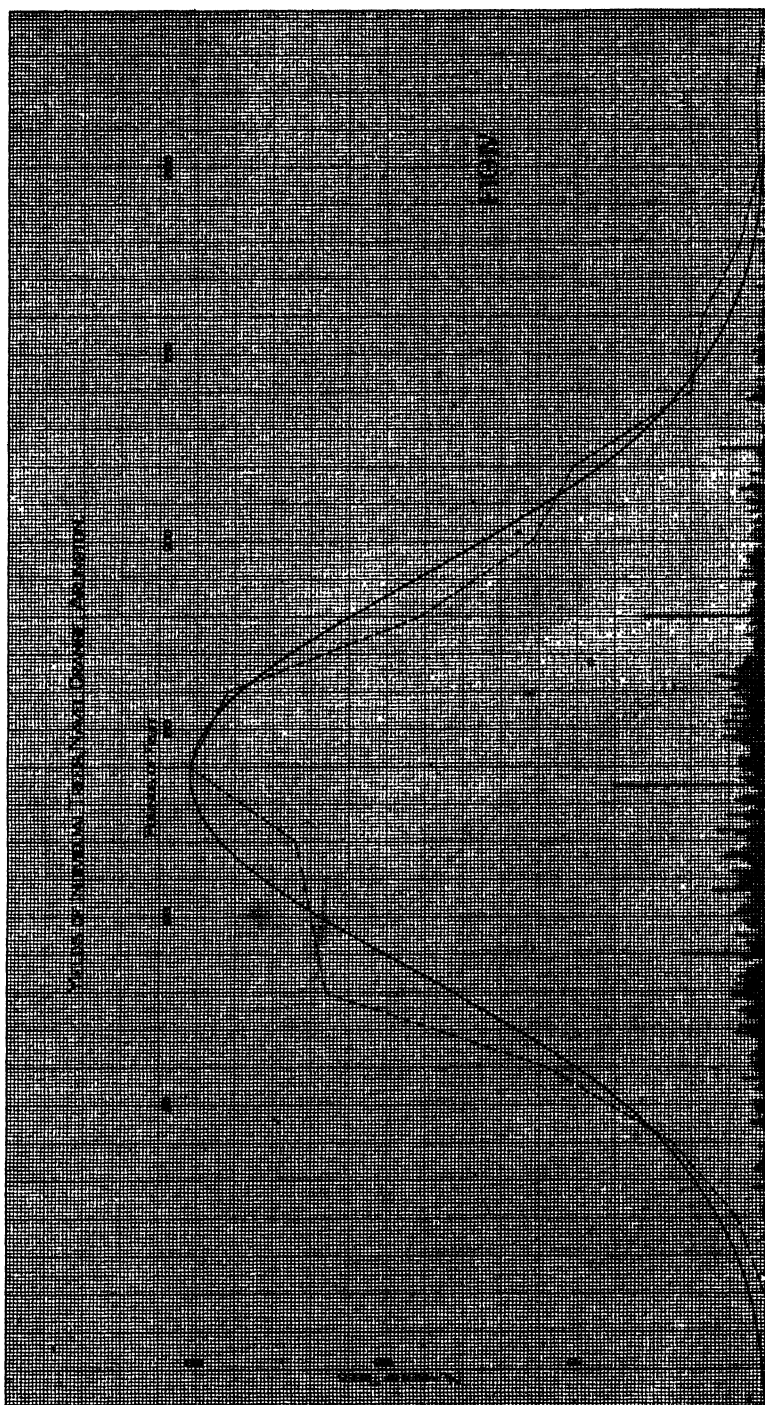
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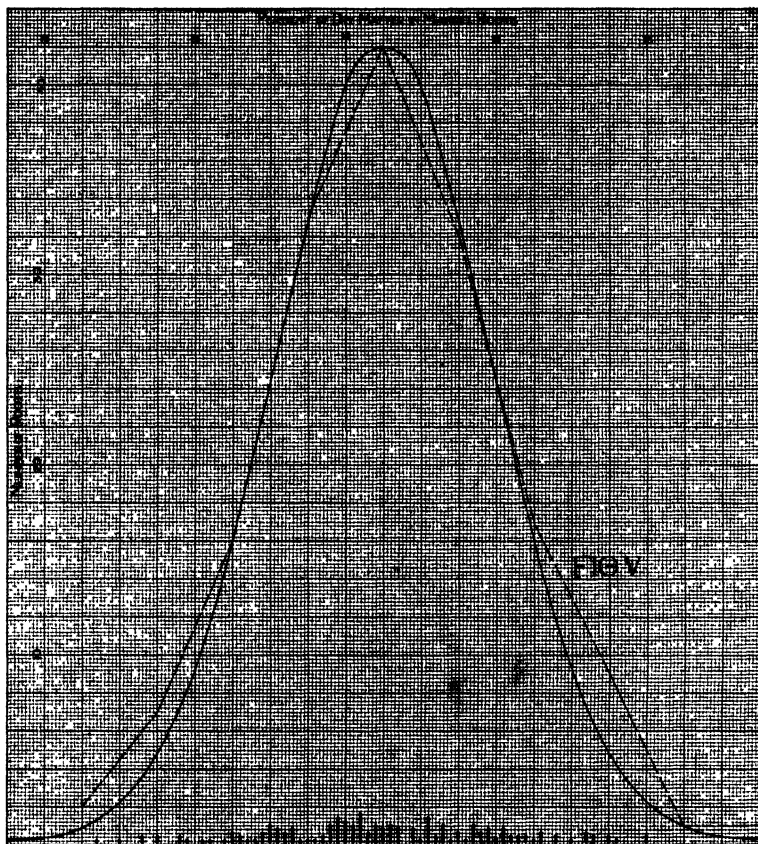


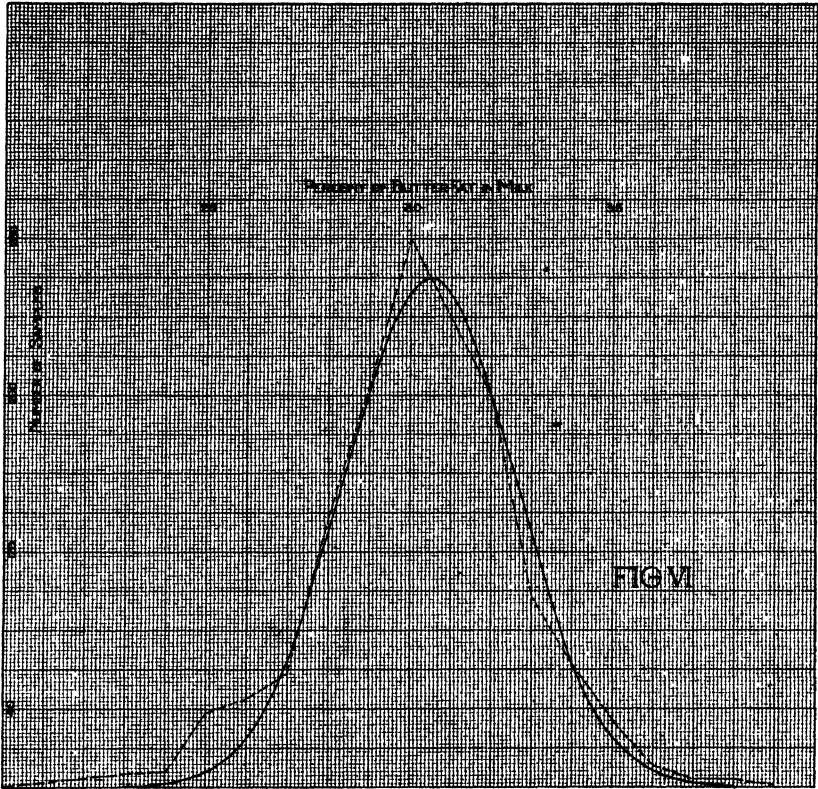


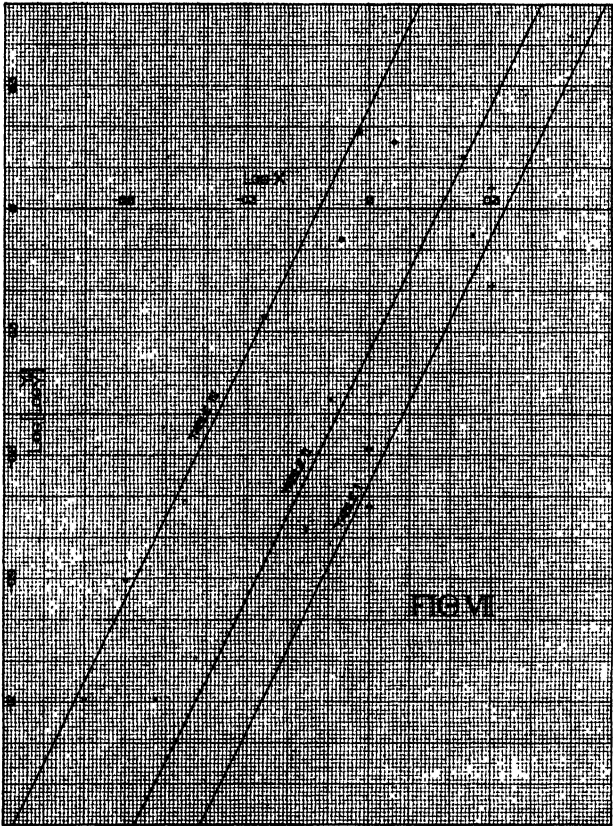


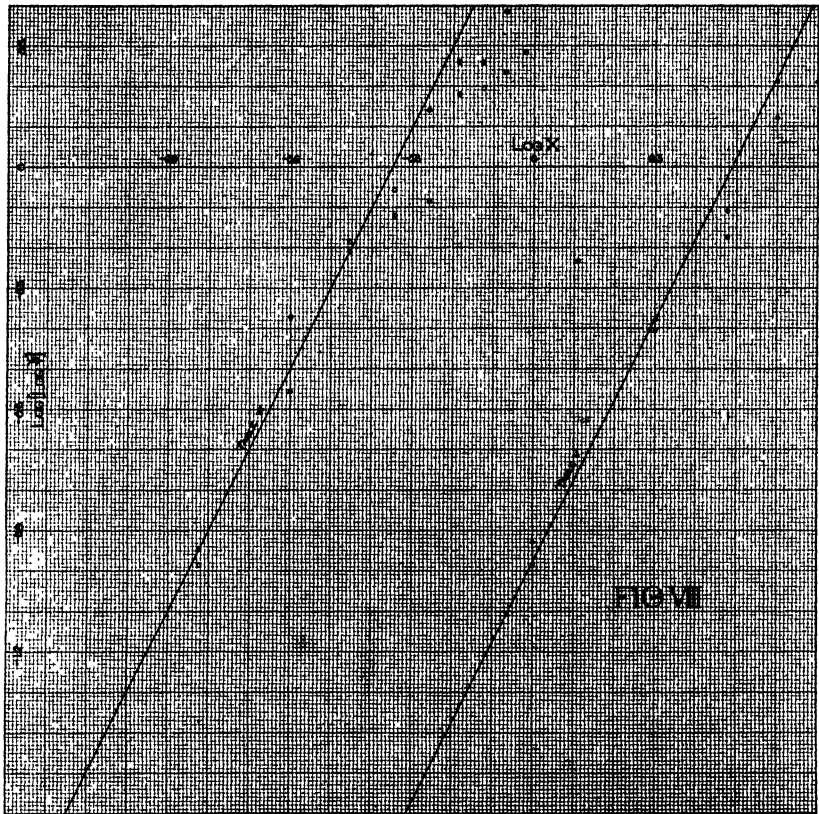


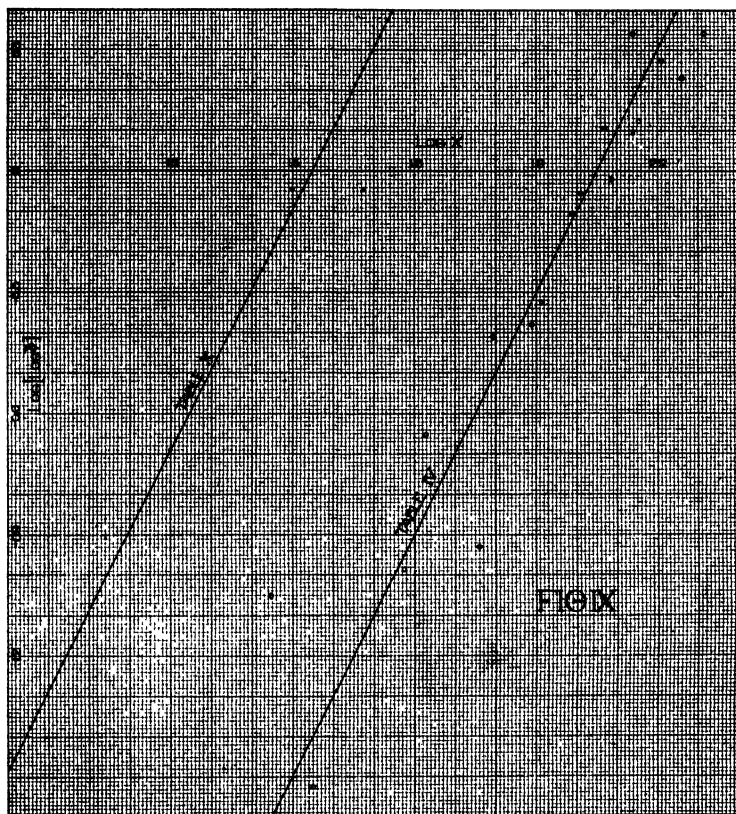


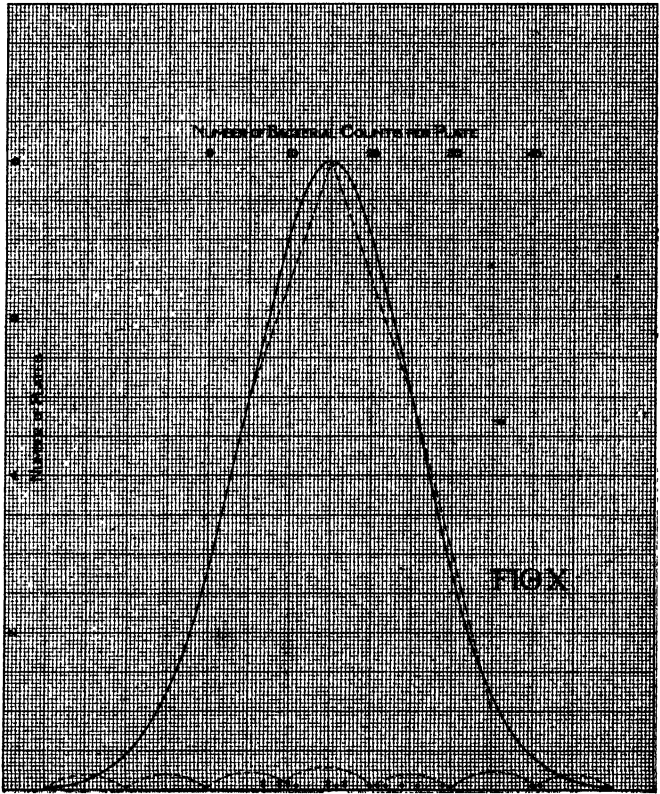


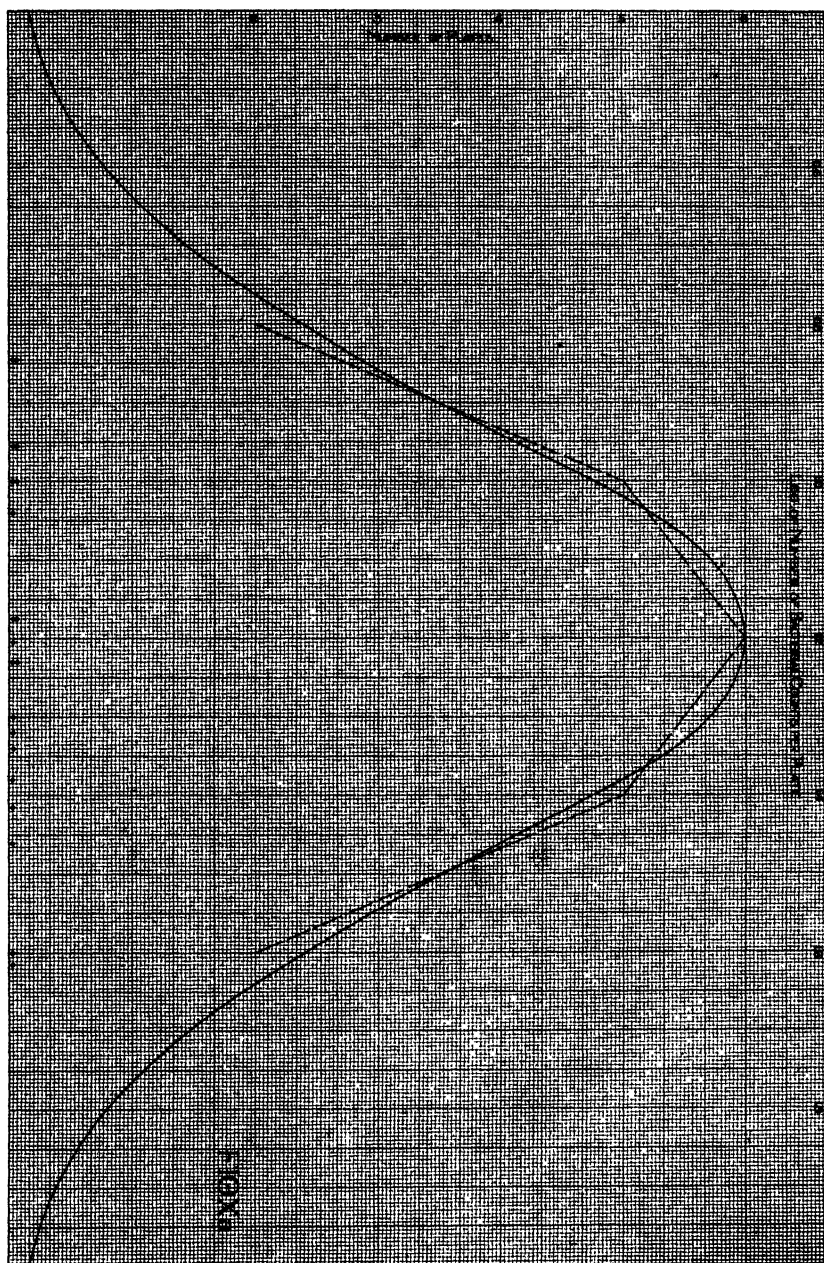












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IN CERTAIN PARASITIC FUNGI

BY

HOWARD S. FAWCETT

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## INTRODUCTION

It is commonly recognized that, of the many different and varying conditions that affect life processes, temperature is one of the most important. The range of temperature at which certain important physiological processes may occur at all is relatively narrow, and comparatively slight temperature changes produce marked effects upon the velocity of other processes having more extended ranges. Although many biological investigators recognized the great importance of this subject, the more detailed study of the effects of maintained temperatures on vital processes awaited the development of simple, adequate and inexpensive methods of artificial temperature control. In the earlier investigations of temperature effects upon organisms it was often impossible to maintain the desired constant temperature throughout sufficiently long periods of time to get results that might be considered as related to maintained temperatures. In recent years a rapidly increasing number of papers reporting investigations on the effects of maintained temperatures upon different physiological processes is an indication that more attention is now being given to this subject. There is still, however, a great lack in our knowledge in this field, especially as regards plants. On certain animal processes somewhat more work appears to have been done, though even in this field much remains to be accomplished.

It should be remembered in this connection, also, that the subject of the temperature responses in living things involves problems more complicated than those just suggested as having to do with *maintained* temperatures. Most organisms (aside from warm-blooded animals) are never exposed, in nature, to maintained temperature for any considerable period of time; their temperature environment is practically always in a state of flux. From this it follows that a knowledge of the relation holding between maintained temperatures and vital processes, no matter how thorough such knowledge may be, can not be expected to be a complete basis for an interpretation of physiological processes going on under natural conditions. In order to obtain a more adequate basis for such an interpretation suitable methods need to be devised for dealing also with *rate of temperature change* as an environmental condition, aside from the degree of temperature itself. The experimental aspect of this phase of physiological and ecological

temperature relations remains practically untouched as yet. It is almost unmentioned in the literature as a serious consideration, although MacDougal (1914) has called attention to its great importance. It is clear, at any rate, that the problem of temperature influence upon organisms falls readily into two fundamentally related but superficially different portions, one dealing with *maintained* temperature and the other with *fluctuating* temperatures. Practically all the controlled experimental work hitherto published deals with the first portion of the problem, and it is in this same category that the present investigation lies. Indeed it seems unwise to attack the problems related to fluctuating temperature until a more thorough appreciation has been gained concerning the general principles underlying the influence of maintained temperatures upon vital processes. It was with the aim of throwing additional light on some of the principles underlying the effects of maintained temperatures on the growth of certain fungi that the investigation reported in this paper was undertaken.

Filamentous fungi were used because they are comparatively simple organisms whose growth rate may be easily measured, because they lend themselves readily to culture in darkness and because, each cell being in direct contact with all features of its environment, their relation to their surroundings is simple and close. The four forms—*Pythiacystis citrophthora* Smith and Smith, *Phytophthora terrestris* Sherbakoff, *Phomopsis citri* Fawcett and *Diplodia natalensis* Evans—were used, all of them being parasitic on citrus trees. These were known to grow well on certain prepared media and some evidence was at hand showing that they differed from one another as regards their temperature relations.

Another reason for selecting these four citrus parasites was the possibility that their pathogenic activities might be influenced by climatic temperature conditions. It was thus possible that a study of their temperature relations might throw some light upon their probable occurrence and upon methods of combating them. General observations in connection with many diseases due to plant parasites have indicated that temperature is a very important factor in their prevalence in any given season or in any given region. N. E. Stevens (1917) has shown that the rate of increase in diameter of chestnut blight cankers is closely related to temperature. Edgerton (1915) has emphasized the apparent relation of temperature conditions to the occurrence of certain plant diseases in subtropical climates. He

is convinced that the absence of anthracnose in beans grown at certain seasons in Louisiana is due to the fact that the average temperatures for the seasons when the disease is absent are too far above the optimum for the growth of the pathogenic fungus. The writer (1917) has previously referred to the limited geographical distribution of melanose due to *Phomopsis citri*, one of the fungi here studied, and has suggested that temperature conditions may be among the important factors limiting its distribution. Humphrey (1914) came to the conclusion that temperature differences in various localities in the state of Washington largely determined the differences in distribution and severity of the tomato wilt induced by *Fusarium oxysporum*. Tisdale (1917) has shown for *Fusarium* wilt of flax that the temperature at which the host is most injured by the disease corresponds to that favoring the maximum growth of the parasite in cultures.

For many parasitic organisms it is probable that the temperature range within which serious infection of their hosts may occur naturally is comparatively small. Temperature differences and differences in moisture conditions may largely account for many of the striking differences observed in the occurrence of many plant diseases from season to season and from one region to another. Many other observations aside from those given above might be mentioned in this connection, but it seems to be clear enough that the pathological or agricultural point of view demands much more thorough knowledge than we yet have concerning the temperature relations of parasitic fungi. It was thus thought that the results obtained in the present study might ultimately be of value in pathological work.

Considering the limited time available for this study, it appeared better to confine the experimentation to the four forms mentioned above and to subject the results to a critical study than to include a larger number of forms, with the accompanying necessity of treating the results in a more superficial manner. Our knowledge regarding the physiology of fungi, as well as that regarding plant temperature relations, may be increased first by intensive studies of a few forms. After the main principles have been worked out for certain selected forms it may become largely a matter of routine to compare a large number of organisms with respect to the principles previously worked out. The four fungi here to be considered seemed to offer opportunities for intensive study, and they also furnish valuable material for comparisons.

As naturally follows from the general concept of conditional control of physiological processes (Verworn, 1912), the relation of the process studied to any given condition is determined not only by the given condition but also by the remaining conditions. For example, if the temperature relations of a given organism are to be dealt with they must necessarily be stated together with as definite a description as possible of the non-temperature conditions that are supposed to be effective. To state that the mycelial mat of a given fungus was observed to enlarge more rapidly at one temperature than at another means little, unless it be also stated just what sort of medium was employed; just what was the length of the time period; just what relation this time period had to the beginning of the test; just what the radiation conditions were, etc. By altering the non-temperature conditions the relations of a given process to different maintained temperatures may be profoundly altered. To illustrate still more concretely: Lehenbauer (1914) found that the optimum temperature for elongation of the shoots of maize seedlings in his experiments was 30° C. when the exposure period was 6 hours, and the corresponding optimum temperature for an exposure period of 12 hours was 32° C. If Lehenbauer's twelve-hour period of exposure be divided into four periods of three hours each, and if the optimum temperature be calculated from his data for each of these four successive periods separately, the optima are found to be 30°, 31°, 31°, and 32° C. respectively. Obviously, any physiological process must be regarded as controlled by all the effective conditions acting together. The conditions that influence the rate of growth of a fungus in a culture may be roughly classified in five groups as follows:

(1) *The nature of the fungus*, which implies its internal conditions—everything that goes to make it the particular organism that it is. This set of internal conditions is vaguely and partially indicated by the name of the fungus, with an implied morphological concept of its form and development, to which the name refers. But it is well known that the same species of fungus may develop quite different complexes of internal characteristics under different sets of environmental conditions. For this reason it is of the greatest importance to include not merely a morphological description but definite information concerning the previous history of the experimental organisms.

(2) *The nature of the medium*, implying all the physical and chemical properties of the space about the hyphae, their environment. For

the most part, the conditions of the medium (aside from temperature and radiation) involve the concentration of numerous chemical substances such as oxygen, carbon dioxide, starches, sugars, acids, inorganic salts, etc.

(3) *Temperature conditions.* Since the temperature of the hyphae follows closely that of the medium and since the latter follows closely the temperature of the more distant surroundings of the culture, it is conventional to consider the temperature of these surroundings as constituting a condition in itself. After all, however, it is the temperature of the fungus hypha that directly influences its rate of growth, not that of the medium, culture dish or chamber about the dish, etc. But, since the temperature of all these spaces is practically the same, this last distinction has generally been ignored. The temperature conditions for two cultures may differ in several ways. If they are maintained temperatures, they may differ in degree or intensity alone, and we may express them in terms of degrees on some thermometer scale. If they are not maintained temperatures, they may differ (a) as to the particular temperatures with which the cultures were started,<sup>1</sup> (b) as to the direction of variation during a given period (whether the temperature became higher or lower with time), and (c) as to the time rate of temperature variation. It is clear that this rate of change in temperature may itself be constant, or may vary throughout a given time period. When only maintained temperatures are to be considered, as in the present study, the only differences to be dealt with between any two cultures are those of degree or intensity as measured in terms of centigrade, etc., degrees.

(4) *Radiation conditions,* involving the various groups of wavelengths of radiation and the relative and absolute intensities of each group. Up to the present time most biological discussion has ignored most of the wave-lengths of radiation excepting the small group commonly designated as *light*. Since the cultures of the present study were uniformly carried out in darkness and in chambers around which a mass of water was continuously circulating, radiation conditions will not require attention here.

(5) *The duration condition,* implying the length of time during which the organism is subjected to the other conditions. From one point of view every condition has a duration factor, but when most of the conditions are maintained, or practically so, the duration factor is common to all, and we may regard it as a separate condition. Moreover, as far as the present investigation is concerned, this duration

condition may be divided into two parts, each one of which may be considered as a separate condition: (a) the actual length of any interval of time considered, and (b) the location of this time interval in the entire culture period reckoned from its beginning. If the time period be always reckoned from the beginning, the second aspect of the duration condition may be neglected and only the length need be considered, as is done in the first part of the discussion of the present investigation. When, however, changes in rate of growth are studied with reference to the age of the culture, the location of the observation period within the culture period, as well as its length, come to be important, and these may be regarded as two different duration conditions, as is done in the latter part of the discussion to follow.

To illustrate all these conditions in detail, a certain fungus, *Pythiaecystis* (condition 1) is surrounded by nutrient agar (condition 2), and subjected to a maintained temperature of 23° C. (condition 3), in darkness (condition 4), and it exhibits an average growth rate of 8.0 mm. per day for a period of three days after inoculation (condition 5).

In the example just given, the observation period begins with the beginning of the culture period. An observation period, however, need not begin with the beginning of the culture period and may not be continued to the end of the culture period. Thus two observation periods may be alike in length, say two days, but they may still have entirely different relations to the beginning of the culture period, so as to constitute, in a sense, distinct duration conditions. Of course, this state of affairs is to be related to changes that go on *within* the organism, with the lapse of time, even though all physical and chemical environmental conditions are assumed to be maintained without alteration. The organism is generally not exactly the same at the moment of inoculation of a culture as it is a day later, four days later, etc. This consideration introduces one of the most perplexing features of the whole study of maintained temperatures as related to vital processes, and considerable attention will be devoted to it in the later sections of this paper.

From the points mentioned in the preceding paragraphs it is, of course, clear that no very definite knowledge of the various environmental influences, as they act to control the physiological processes of any organism, may be expected from physiological tests in which any of the effective conditions are allowed either to vary or to differ in unknown ways. As long as the conditions differ only in known



ways from one culture to another, or as long as they vary only in *known* ways in the same culture, there is hope of advancing our knowledge of environmental influences.

In order that the inoculum for each series should be as similar as possible to that of any other series, the four species were kept in the dark in stock tube cultures with ordinary corn-meal agar and at a temperature ranging from about 16° to 18° C. From these primary stock cultures inoculations were made at frequent intervals, on agar plates of the same kind of medium and kept at the above temperature range. These plates formed the secondary stock cultures. The marginal region of a mycelial disk of a secondary stock culture (about five days after inoculation) furnished material for inoculating the experimental cultures of that fungus. The inocula for each species were fairly similar, therefore, with respect to parentage, age, vegetative activity, etc. Practically the same amount of inoculating material was always transferred to each experimental culture. It is consequently safe to suppose that all experimental cultures of the same fungus were practically alike at the beginning, no matter when they were made. The four fungi used furnish, for the whole study, four different sets of initial complexes of internal genetic conditions. Progressive variation in the internal conditions of the fungus is one of the features taken into consideration and will receive attention in later sections.

Although several different media were employed in certain aspects of the experimentation, only one (corn-meal agar) will be considered in the present paper. Special precautions were taken to have this medium as nearly as possible the same at the beginning of all cultures, no matter at what time they were started. The consistency of results obtained by repetition showed that this aim was practically attained. It was also shown by special tests on one of the fungi (*Pythiacystis*) that the unoccupied medium did not considerably alter during the period of any single culture. It therefore seems safe to suppose that the medium was always the same at the beginning of all cultures, and also that the medium remained practically unaltered during the progress of any culture, at least until it was reached and passed by the enlarging web of hyphae. Unquestionably the medium occupied by the mycelial disk suffered alterations in composition, but it was not apparent that such changes influenced the rate of growth of marginal hyphae.

Since the elongating hyphae lie largely near the aerial surface of the agar plate, while some are partially in contact with the air space above, it is well to consider the aerial environmental conditions, as well as those within the agar medium itself. Aside from temperature, the air conditions in the culture dishes above the agar were sensibly the same in all cultures at the start, except that the pressure of water vapor was, of course, different for cultures exposed to different temperatures. Since the air space of the culture dish was always practically saturated with water vapor, the pressure of the water vapor would nearly follow the equilibrium vapor pressure of water at the various temperatures. The unsealed dishes allowed a slow escape of water vapor and, consequently, a slow evaporation from the agar surfaces during the culture period, the rate of water loss being somewhat greater at higher than at lower temperatures. Different maintained temperatures, therefore, were automatically accompanied by slightly different rates of variation in the water content of the yet unoccupied medium. Such variation may be neglected in this case, however, since it was shown by special tests that variations even larger than those that actually occurred in the experimental cultures had no appreciable influence on the rates of growth of the fungi.

As has been said, the temperature conditions were always artificially maintained, with a very small degree of fluctuation throughout any given culture period. The radiation conditions are regarded as nonexistent in these tests. Light (and radiation of still shorter wavelengths) was always excluded and the stirring apparatus operated to prevent any one-sided action of long-wave radiation upon the cultures.

The duration condition offers no particular difficulty in such work as this. Since the experimental cultures are all regarded as alike at the time of inoculation, the duration conditions may be regarded as beginning to operate from the beginning of this culture period, the time of inoculation being considered as zero time. If either the length of the culture period or the time between observations for any culture is different from that for another, this fact is, of course, quantitatively shown by the inoculation intervals between successive observations.

From the preceding discussion it will be observed first that the research at hand was so planned as to involve the actual or assumed control, during the respective culture periods, of all the groups of effective conditions except internal ones, and second, that the only conditions considered as effectively different from culture to culture

are: (1) the nature of the fungus used (initial internal conditions), (2) the rate and direction of physiological alteration within the organism, and (3) maintained temperature.

The following scheme may serve to show the sorts of terms that enter into the interpretative comparisons that may be made for an investigation of this kind:

#### A. COMPARISON OF CULTURES OF THE SAME FUNGUS

- I. Internal conditions (genetic constitution and physiological state of fungus).
  1. Initial conditions, alike for all cultures of same fungus.
  2. Direction and rate of physiological alteration during culture period, may be different from one set of cultures to another. This alteration always to be stated as *within the limits* prescribed by:
    - (a) The initial internal conditions, and
    - (b) The external conditions.
- II. External conditions (environment).
  1. Initial environmental conditions, except temperature, considered alike for all cultures of same fungus.
  2. Initial temperature conditions different from one set of cultures to another set.
  3. All environmental conditions assumed to be maintained at their initial values throughout the culture periods.

#### B. COMPARISON OF CULTURES OF DIFFERENT FUNGI

- I. Internal conditions (genetic constitution and physiological state of fungus).
  1. Initial internal conditions different for four different fungi.
    - (a) As to genetic constitution (whose capabilities are roughly indicated by taxonomic description).
    - (b) As to physiological state, because of different reaction of different fungi to essentially identical preliminary environmental conditions.
  2. Direction and rate of physiological alteration during culture period, may be different from culture to culture. This variation always to be stated as within the given limits set by the initial internal conditions and the external conditions, as above.
- II. External conditions (environment).
  1. Initial environmental conditions, except temperature, considered alike for all cultures.
  2. Initial temperature conditions either alike or different from culture to culture.
  3. All environmental conditions assumed to be maintained at their initial values throughout the culture periods.

The study here reported is thus seen to comprise five different studies. The influence of maintained temperatures on the growth rate of each of four fungi was measured, under the given non-temperature conditions, which are considered as initially alike, and under the given

initial internal conditions also considered as alike for all cultures of the same fungus. The fifth study comprises the comparison of the four fungi as to their temperature relations under the given set of non-temperature, external conditions.

The investigation reported in the present paper was carried out during the period between October, 1916, and June, 1918, in the laboratory of Plant Physiology of the Johns Hopkins University. The author wishes to express his thanks to Dr. H. J. Webber and the University of California for arrangements that made it possible for him to be absent from the Citrus Experiment Station during the period just named. He also wishes to record his appreciation of the privileges and facilities accorded him by the Johns Hopkins University, including a Johnston scholarship in that institution. Finally, he desires to acknowledge his indebtedness to Professor B. E. Livingston and to Dr. H. E. Pulling, of the laboratory of Plant Physiology of the Johns Hopkins University, for much valued aid and criticism in connection with the planning and carrying out of the experimental part of this study and in the interpretation and presentation of the results.

## METHODS

### THE CULTURE MEDIUM

The corn-meal agar employed in these experiments was prepared according to the procedure described by Shear and Wood (1912), using 20 gm. of corn meal and 15 gm. of agar shreds for each liter of water. More water was added before the final filtering, so that there was one liter of the medium for each 20 gm. of corn meal originally used.

The exact chemical and physical nature of such a culture medium can not, of course, be stated. It undoubtedly contains a large number of inorganic salts and a still larger number of organic compounds, all in rather low concentration. It also contains various substances in a state of suspension, and, since it has more or less the nature of a gel, there is no marked tendency for these to precipitate out. Since the time available for this study was limited, it was decided to make no attempt to devise a nutrient medium of known composition or even to find out what any of the media commonly employed by mycologists

may contain. In order to be able to proceed immediately to the problem of temperature influence, the whole matter of nutritional conditions—a very important one in itself—was ignored. All that was done in this connection was to be sure that the medium employed would support what appeared to be excellent growth of all four fungi, and to take precautions so that practically the same medium might always be used throughout the entire study. Since corn-meal agar is an infusion of corn meal and agar-agar shreds, both of them exceedingly complicated, unknown, and variable materials, it was feared that different samples of the medium might be very different. And an attempt was made to avoid this danger by preparing enough medium at the beginning for the entire investigation, mixing it thoroughly in a single container and then preserving it in bottles for future use. That the infusion itself might alter with time was, of course, possible, but various repetitions of the experiments indicated clearly that such alteration—if it occurred—was not of such nature and magnitude as to alter the growth of the fungi when other conditions were the same.

Since the amount of medium necessary for the entire study could not well be prepared in a single day, about eight liters were made at a time, until about 48 liters were ready. The entire amount was then liquefied by heat, placed in a 20-gallon earthenware vessel and thoroughly mixed, after which it was poured into liter bottles. The mouths of the bottles were then plugged with cotton in the usual way and the bottled medium was immediately subjected to a temperature of 115° C. for 15 minutes. This heating was repeated on the two following days, after which the tops of the cotton plugs were flamed and covered with several thicknesses of paraffined paper, tied tightly around the bottle neck. The bottles of medium were stored in darkness at a temperature of about 18° C. When a lot of cultures were to be made the required number of bottles of medium were removed from storage, brought into the liquid condition by heating in the autoclave, and used in the ordinary way for pouring the plates. About 15 c.c. of medium was used in each culture dish. It was found by test that this amount might be increased or diminished by as much as 3 c.c. or more without perceptibly influencing the growth of the fungi.

## STOCK CULTURES

The original sources of the fungus materials were as follows: *Pythiacystis citrophthora*, isolated by the author from the diseased bark of a lemon tree suffering from gummosis, at Whittier, California, in August, 1915; *Phytophthora terrestris*, also isolated by the author from the diseased bark of a citrus tree suffering from *mal di gomma*, at Palmetto, Florida, in January, 1914; *Diplodia natalensis*, isolated (by Mr. J. M. Rogers) from a citrus tree in the Isle of Pines, W. I., and received by the writer in the fall of 1916; and *Phomopsis citri*, received from H. E. Stevens, from Florida, in October, 1916. All four fungi had been cultivated under the same conditions, in tubes of corn-meal agar, in darkness at from 16° to 20° C., for at least nine months previous to the beginning of the experiment. During that time transfers to new tubes of media had been made at intervals of about six or eight weeks. These cultures may be termed the primary stock cultures in this study. The four fungi, therefore, had each been subjected for at least nine months, to the same environmental conditions. They had also been grown in the same kind of medium as that into which they were now to be introduced for the temperature experiments.

## THE EXPERIMENTAL CULTURES

Approximately five days before the starting of each series of experiments several cultures of each fungus were started, by transferring small bits of medium containing mycelium from a primary stock culture to the center of the agar plates. These were the *secondary* stock cultures, and were kept in darkness with a temperature of about 20° C. for about five days previous to the making of the *experimental* cultures. Little plugs or disks were cut out of the agar plate just behind the advancing margin of the circular growth area of one of these five-day secondary stock cultures. The disks were 2.5 mm. in diameter and about 1.5 mm. thick. They were cut out by means of a cylindrical platinum cutting device like that described by Keitt (1915). Each disk was lifted on the flattened end of a platinum needle and was then inverted and placed centrally upon the surface of a new agar plate. The petri dishes used were 10 cm. in diameter and 1 cm. deep; each contained approximately 15 c.c. of corn-meal agar, which had been poured hot and allowed to solidify before the transfers were made. After inoculation the experimental cultures were divided into

seven similar groups, and one of these groups was placed in each of the seven chambers of the temperature-control apparatus. The cultures of any given species always occupied the same relative position in all the chambers and in all the series. This precaution was observed so that any possible difference in temperature between the upper and lower portions of the chamber would not render the measurements of the different lots of the same species incomparable. But such differences in temperature between different parts of any one of the seven chambers proved to be slight (less than  $0.5^{\circ}$  C. between the top and bottom of a chamber). The cultures of *Pythiacystis citrophthora* and *Phytophthora terrestris* occupied a position, on the rack in the chamber, at nearly the same level as the bulb of the thermometer from which the temperature records were taken. The cultures of *Phomopsis citri* were about 15 cm. below and those of *Diplodia natalensis* were about 15 cm. above the thermometer bulb in each case.

#### OBSERVATIONS ON GROWTH

As the hyphae grew out in all directions from the center of the plate a rounded mat or mycelial disk was formed on or near the surface of the medium. This disk remained practically circular, as it enlarged, for both *Pythiacystis citrophthora* and *Phomopsis citri*, forming a nearly perfect circle at all stages of enlargement. The mycelial disks of *Diplodia natalensis* and *Phytophthora terrestris* were often slightly irregular in form or rather evenly lobed, especially at the higher temperatures used. No irregularities in growth, such as bring about zonation in mycelial mats of many fungi, were observed in any of the cultures with maintained temperatures. In special tests, however, in which the fungi were grown for a certain time in one temperature and then transferred and grown in a markedly different temperature, such zonation was pronounced.

Observations were made at daily intervals for a culture period of from four to six days. The chief matter of observation was the mean diameter of the disk, which was obtained by averaging two measurements of different diameters, selected to represent the disk as a whole. When the margin of the enlarging disk was clear and definite these measurements were made by means of a thin millimeter scale applied on the bottom of the Petri dish outside. In other cases the Petri dishes were inverted and the length of the mycelial outgrowth was measured by means of a microscope with an ocular micrometer.

Measurements with the millimeter scale were read to within 0.5 mm. This was deemed sufficiently precise for the purpose.

Since none of these fungi produced anything but vegetative hyphae during the culture periods employed and the growth activities were not complicated by the formation of any reproductive bodies, these measurements of the mycelial disks and the daily increments of disk enlargement derived from them appear to furnish as satisfactory a criterion of physiological activity in general as might be found. The only other criterion for such comparisons as these, and that hitherto generally used by physiological workers, is the rate of production of mycelium measured on the basis of dry weight; the employment of this criterion offers great practical difficulty when agar medium and short culture periods are used.

At the time of observations, each chamber was opened for a fraction of a minute to remove just one group of cultures, all the cultures being alike. These dishes were immediately wrapped in cotton batting, to exclude light and prevent very rapid temperature changes. Each dish was removed from the wrapping for a minute or less, while the observations were made, and was then returned to the wrapping. After all the cultures of the group had been observed the entire group was replaced in its temperature chamber and another group was taken out for observation. The time required for the entire operation of removing, measuring, and replacing a group of 10 cultures averaged less than 10 minutes.

The opening of the chambers for removing and replacing the groups of cultures had very little effect upon the temperature of the chamber itself. The thermographs in the chambers usually showed the occurrence of this series of momentary openings by a slight rise or fall of the pen tracing, producing short vertical lines, each representing a degree or less of practically momentary alteration in the temperature of the chamber.

Several tests were carried out to determine whether the daily disturbance of the maintained temperature, caused by removing the cultures for observation, might exert any appreciable influence on the growth of the fungi. These tests showed that the amount of growth observed after several days was practically the same whether the cultures had been left in the chamber for the whole period or had been removed for daily observation in the regular way. These daily disturbances of the maintained temperature are considered negligible in the present study.



### THE MAINTAINED TEMPERATURE CHAMBERS

The various temperatures employed in the experiments here considered were maintained by means of an apparatus described by Livingston and Fawcett (1920). This apparatus consisted essentially of seven experiment chambers about 33 cm. in diameter and 43 cm. deep, each one surrounded below and at the sides by a large mass of water. Light was excluded. The air of the chamber and the water around it were kept in constant circulation by mechanical stirrers. The seven chambers were built in a row, with the water jacket of each in contact with that of the next, except for a sheet-iron partition which kept the several masses of water entirely separate. A tank of mechanically stirred water having automatic temperature control was added at either end of the series of experiment chambers and the entire apparatus was well insulated from the surroundings. The two ends of the series were adjusted for any two desired temperatures. Between these, after equilibrium had been established, lay the maintained temperatures to be studied, each of which differed from the next by a certain amount, depending on the position of the chamber in the series. The daily fluctuations in any chamber were only rarely more than  $0.5^{\circ}$  C. Access to the chambers was had from above, and, of course, the maintained temperatures of the cultures were slightly disturbed by opening for observations, as has been noted.

### STRUCTURAL DIFFERENCES RELATED TO TEMPERATURE

Microscopic observation of the fungus hyphae near the margin of the mycelial disk was made occasionally, at the time of measuring. Since no spores were produced in any of the experimental cultures in the time here recorded, vegetative growth alone can be considered. The only structural differences observed between different cultures of the same fungus consisted in more or less marked peculiarities in cultures that had been exposed to very high or very low temperatures. Within a range of maintained temperatures extending downward about 12 degrees or 15 degrees centigrade below a temperature slightly above the optimum temperature for enlargement no influence of temperature on structure was noticeable. Within this range the hyphae were of regular and simple form and the branching was regular.

With temperatures near the maximum point at which any enlargement would occur, the outgrowing hyphae were of irregular shape, bent and twisted, with occasional swellings and usually with apical enlargements. The hyphal diameter was usually much larger than that of hyphae with more favorable temperatures for enlargement, and these thicker irregular hyphae showed contents that appeared dark colored and granular, in contrast to the smooth, clear appearance of the cell contents for cultures with the more favorable temperatures for enlargement. The granulation was frequently pronounced and refraction was such as to give the whole hypha a very dense appearance.

With the lowest maintained temperature tested ( $7.5^{\circ}$  C.) the hyphal diameter for *Pythiacystis* and *Phytophthora* was much greater than with temperatures within the favorable temperature range. The hyphal contents for these low-temperature cultures was only slightly granular. The low-temperature filaments of *Pythiacystis* were much swollen and were divided into many short, thick, club-shaped branches; those of *Phytophthora* showed a series of swollen joints. These low-temperature cultures of *Phomopsis* showed filaments somewhat smaller in diameter, with less frequent branching, than those of cultures grown with favorable temperatures for rapid enlargement. In *Diplodia* the diameters of the hyphae were also somewhat smaller at  $7.5^{\circ}$  C. than at more favorable temperatures for enlargement.

## THE EXPERIMENTAL DATA

As previously noted, the temperature apparatus contained a battery of seven chambers, so that seven different maintained temperatures could be employed at one time for a given series of cultures. After preliminary tests the apparatus was so adjusted as to give the maintained temperatures that promised to be most useful. Two diameters of each mycelial disk were measured at the end of each 24-hour period during the experiment. The average of these two measurements was taken to represent the average diameter for a given culture at the time of measurement. From this average diameter at the end of the first 24-hour period the diameter of the transplanted cutting (2.5 mm.) was subtracted and the remainder was taken as the value for the increment of enlargement for this first observation period. The difference between the average measurement for the end of the first and that for the end of the second 24-hour period represented the

increment of enlargement for the second 24-hour period. Increments for the subsequent periods were obtained in a similar way. All the culture averages for a group of like cultures in the same chamber were finally averaged to give the *group mean*. These group means were taken as relative measures of the rates of radial enlargement for the different fungi, different time periods, and different maintained temperatures.

In tables I-IV are presented the group means for the four fungi, for the different maintained temperatures, and for the different 24-hour observation periods, as well as for the first 2-day and the first 3-day periods. In cases where a considerable number of data are at hand for culture periods longer than three days the group means are given for the first 4-day, first 5-day, etc., period after inoculation. The number of cultures employed in the derivation of these group means is also indicated in each case in parentheses. The rates given in tables I-IV do not always represent single series. In many cases the same maintained temperature was tested at different times for the same fungus, and all the measurements available for any fungus and temperature have been used in deriving the mean rate for that fungus and temperature, without reference to the particular series of tests in which any group of measurements may have occurred. Also, the data in any vertical column of these tables, representing the enlargement rates for the respective maintained temperatures indicated in the first column, do not all represent the same series.

Thus a test for a given maintained temperature may have been made in July and repeated in August and the two sets of data combined for the particular fungus and temperature in question. Many repetitions of this sort were made, involving the same maintained temperature in different experimental series, and the growth rates of similar cultures in different series usually agreed as closely as did those of duplicate cultures of the same series. This indicated that the initial fungus materials and nutrient medium used did not appreciably alter during the period of the investigation. It will be noticed that the number of cultures (shown in parentheses) after each rate in the first part of the tables usually decreases after the second or third consecutive exposure period. This is due to the fact that some of the cultures in each temperature chamber were transferred to other chambers with a different temperature after the second or third day, and the subsequent growth increments measured. These data are intended for a later paper and are not included in the present discussion.

TABLE I  
MEAN 24-HOURLY DIAMETER INCREMENTS (MM.) OF MYCELIAL DISKS FOR PHYTHIACYSTIS CITRIPHOTHA  
(Numbers in parentheses indicate number of cultures from which mean was obtained.)

[illegible]

TABLE II  
 MEAN 24-HOURLY DIAMETER INCREMENTS (MM.) OF MYCELIAL DISKS FOR PHYTOPHTHORA TERRESTRIA  
 (Numbers in parentheses indicate number of cultures from which mean was obtained.)

Maintained temperature, deg. C.	First 24-hour period	Second 24-hour period	Third 24-hour period	Fourth 24-hour period	Fifth 24-hour period	Sixth 24-hour period	First 2-day period	First 3-day period	First 4-day period	First 5-day period	First 6-day period
7.5	.02 (10)	.14 (10)	.21 (10)	.7 (7)	.8 (5)	.9 (5)	.08	.12	.27	.37	.46
13.5	1.0 (10)	2.0 (10)	2.3 (10)	3.2 (7)	4.4 (5)	5.6 (5)	1.5	1.76	2.12	2.58	3.08
15.5	1.1 (8)	3.2 (8)	5.6 (8)	5.4 (4)	4.7 (4)	-----	2.15	3.3	3.82	4.0	-----
18.5	2.5 (12)	6.3 (12)	6.4 (12)	8.2 (10)	9.1 (8)	9.7 (3)	4.4	5.06	5.8	6.5	7.03
21.5	3.6 (9)	8.1 (9)	8.6 (9)	9.0 (9)	9.1 (9)	9.1 (9)	5.85	6.76	7.32	7.68	7.9
23.0	4.0 (8)	9.6 (8)	9.8 (8)	9.8 (4)	9.8 (4)	-----	6.8	7.8	8.3	8.6	-----
24.5	4.2 (10)	10.5 (10)	11.3 (10)	11.4 (6)	11.0 (3)	11.0 (2)	7.35	8.66	9.35	9.68	9.9
27.0	5.3 (9)	11.0 (9)	11.7 (9)	11.0 (4)	10.8 (4)	-----	8.15	9.33	9.75	9.96	-----
30.0	5.5 (10)	13.8 (10)	13.3 (10)	13.2 (10)	10.9 (6)	10.7 (3)	9.65	10.86	11.45	11.34	11.23
31.0	5.8 (2)	13.1 (2)	13.1 (2)	13.0 (2)	11.4 (2)	-----	9.45	10.66	11.25	11.28	-----
32.0	6.3 (8)	13.6 (8)	13.7 (8)	14.0 (4)	11.0 (4)	-----	9.95	11.2	11.9	11.72	-----
34.5	7.3 (11)	8.6 (11)	11.0 (11)	-----	-----	-----	7.95	8.96	-----	-----	-----
35.5	4.8 (2)	4.2 (2)	2.6 (2)	2.5 (1)	0 (1)	-----	4.5	3.86	3.52	2.8	-----
36.0	1.0 (10)	.5 (10)	.3 (10)	.2 (2)	0 (2)	-----	.75	.6	.5	.4	-----
36.5	.8 (2)	.6 (2)	0 (1)	-----	-----	-----	.7	.46	-----	-----	-----
40.0	0 (10)	0 (10)	0 (10)	0 (8)	0 (6)	-----	-----	-----	-----	-----	-----
45.0	0 (10)	0 (10)	0 (10)	0 (8)	0 (6)	-----	-----	-----	-----	-----	-----



TABLE IV

MEAN 24-HOURLY DIAMETER INCREMENTS (MM.) OF MYCELIAL DISKS FOR  
*DIPLODIA NATALENSIS*

(Numbers in parentheses indicate number of cultures from which mean was obtained.)

Maintained temperature, deg. C.	First 24-hour period	Second 24-hour period	Third 24-hour period	First 2-day period	First 8-day period
7.5	.05 (10)	1.9 (10)	2.1 (10)	.97	1.35
13.5	8.0 (10)	11.0 (10)	10.0 (10)	9.5	9.66
15.5	10.1 ( 6)	13.7 ( 6)	14.2 ( 6)	11.9	12.66
18.5	13.0 (12)	17.5 (12)	18.5 (12)	15.25	16.33
19.5	15.5 ( 8)	20.5 ( 8)	18.5 ( 8)	18.0	18.16
21.5	17.0 ( 8)	21.5 ( 7)	23.5 ( 7)	19.25	20.66
23.0	18.2 (22)	24.0 (22)	23.7 (22)	21.1	21.96
25.0	23.0 (10)	27.2 (17)	26.0 (17)	25.1	25.4
27.5	25.9 (18)	31.0 (18)	26.1 (18)	28.45	27.66
29.5	27.7 ( 9)	29.3 ( 9)	24.5 ( 9)	28.5	27.16
30.0	30.0 (12)	29.8 (12)	23.6 (12)	29.9	27.8
31.0	29.3 ( 7)	25.5 ( 9)	21.5 ( 9)	27.4	25.43
32.5	27.5 ( 7)	25.0 ( 7)	21.1 ( 7)	26.25	24.53
34.0	26.0 ( 8)	21.5 ( 8)	18.0 ( 8)	23.75	21.83
35.5	14.5 (10)	5.0 (10)	0 (10)	9.75	6.5
36.5	9.1 (15)	1.0 (15)	0 (15)	5.05	3.36
40.0	1.5 (15)	0 (15)	0 (13)	.7	.5
41.0	.7 ( 8)	0 ( 8)	0 ( 8)	.35	.23
45.0	.2 (10)	0 (10)	0 (10)	.1	.06

## DISCUSSION OF RESULTS

## GENERAL CONSIDERATIONS

It is obvious from an examination of the data in tables I-IV that the magnitude of the mean rate of enlargement of mycelial disks (here expressed for convenience in terms of the diameter increment per time period of 24 hours) is influenced by the variations in at least three conditions for any one organism, namely, (1) the degree of maintained temperature, (2) the length of the time period considered in deriving the mean rates, and (3) the time relation of any given observation period to the beginning of the entire culture period. As has been pointed out, each number in the first column represents a given temperature nearly constantly maintained over the entire period indicated in the tables. The diameter increments given in the remaining columns may be considered as rates, expressed in millimeters per 24 hours or one day. In the first part of the tables the rates of

diameter increment (mm. per 24 hours) for any one temperature are for consecutive exposure periods, also of 24 hours in length, the consecutive columns representing the manner in which the increment rates change from one 24-hour period to the next, etc., with the lapse of time. In this case each exposure period has a different time relation to the initial moment of exposure to the given temperature, i.e., each exposure period began where the preceding period terminated. In the second part of the table the consecutive columns show the mean daily increments (mm. per 24 hours) for exposure periods of different length (2 to 6 days), but each having the same time relation to the beginning of the culture period, the time period always beginning with the beginning of the culture period. The rates derived in the manner shown in the second part of the tables are here included in order to show the manner in which the relation between growth and temperature has been worked out in some of the previous investigations with plants in which minimum, optimum, and maximum temperatures for growth have been considered. It was in connection with the employment of such time periods as these that Lehenbauer (1914) discusses the growth-temperature relations for shoots of maize seedlings.

It is readily seen from an examination of these tables that because of the influence of the time factor the old conception of a definite optimum temperature for growth of a given organism is inadequate. Blackman (1905) pointed out that the term "optimum temperature" as commonly used had no definite meaning. Lehenbauer, in order to make the term "optimum temperature" usable, defined it as *that temperature at which there is best growth during a given time period*. In this sense a process may have, not one, but a large number of temperature optima. Blackman states in connection with his discussion of carbon dioxide assimilation that the time factor is important only at higher temperatures, the higher the temperature the more rapid the falling off of the rate with time. A new definition of optimum temperature, based on this idea, has been proposed by Leitch (1916), namely, the *highest temperature at which no time factor enters*. Since the time factor may be operative at all temperatures at which growth is possible for some organisms, the optimum so defined would have no important meaning for such organisms. For convenience of discussion, Lehenbauer's definition will be followed in this paper. An added restriction, however, is to be placed on this definition when the growth rates for consecutive observation periods are to be considered. An examination of the first part of tables I-IV shows that in order



to define the optimum for such data not only must the length of the observation period be stated but the relation of that time period to the beginning of the culture period must also be given. Lehenbauer's observation periods, from which his rates were derived, all had the same relation to the beginning of the culture period.

Turning again to the first part of table I-IV, it is evident that there are three variables to be considered. The *growth rate*, expressed in millimeters per 24 hours; *temperature*, expressed in centigrade degrees, and *time*, expressed in number of days from the beginning of the culture period. The relation between these three varying quantities can best be discussed for our purpose by means of graphs and the graphs used will be of three kinds: (1) those showing the relation between growth rate and temperature at fixed time periods, (2) those showing the relation between growth rate and the march of time from the beginning at given maintained temperatures of the culture, and (3) those showing the relation of the magnitude of the temperature coefficient ( $Q_{10}$ ) to the shifting of the 10-degree temperature intervals from which the coefficients are derived.

The relations of these three variables (rate of growth, temperature, and time) could, of course, all be represented graphically together by means of lines on a drawing showing three dimensions, as is done by Rahn (1916) for rate of development of bacteria with temperature and time. While this is the most complete manner of showing the relation existing between these three variables, making clear at once the uselessness of considering any growth-temperature relation without reference to the influence of time, it is not so convenient for our present discussion as is the use of a number of simple graphs.

### THE GROWTH-TEMPERATURE GRAPHS

The growth-temperature graphs were constructed in the ordinary way. For the given fungus and observation period the mean 24-hour rates (first part, table I-IV) were plotted as ordinates and the indices of maintained temperature were plotted as abscissas. After the points were in place a smoothed graph was drawn in the regular manner.

To illustrate this process of smoothing, the four graphs for the second 24-hour period after inoculation are shown together in figure I. The points shown on or near each smoothed graph represent the mean rates taken from the table. It is at once seen that they arrange themselves in a very satisfactory manner as regards the smoothed graph,

i.e., that the process of smoothing introduces only very slight alterations from any of the values derived directly from observations. These four second-day graphs are representative of the others. All are shown (without the points, the values for which may, however, be obtained from tables I-IV) in figures 2-5, each figure presenting

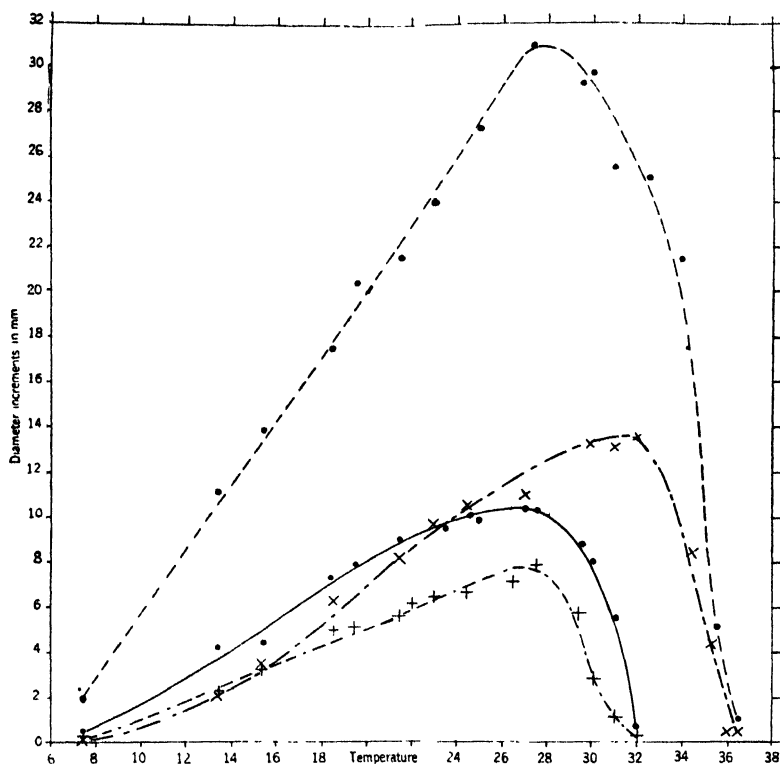


Fig. 1. Smoothed growth-temperature graphs for the second 24-hour period for each of the four fungi employed. The points represent the actual increments as given in tables I-IV.

*Phythiacystis citrophthora* —————  
*Phytophthora terrestria* - - - - -  
*Diplodia natalensis* - - - - -  
*Phomopsis citri* - - - - -

the several smoothed graphs for a single fungus. These graphs represent the growth-temperature relations for each one of the successive 24-hour observation periods (within the exposure period) for which adequate data were available.

In general form and shape the growth-temperature graphs of the four fungi are much alike. Beginning with the lowest temperature tested, the graphs all rise gradually, being slightly concave upward

at first, but becoming decidedly convex upward as the graph maximum is approached. Beyond this maximum region the graphs descend rapidly to the graph minimum (maximum temperature for growth). It is clear that the growth optimum always lies far above (to the right of) the middle of the total temperature range and that the upward slope of every graph is much less steep than the downward slope.

In these general characteristics these graphs resemble those of Edgerton (1915) for the growth of *Glomerella*, those of Lehenbauer (1914) for the growth of maize seedlings, those of Leitch (1916) for the growth of roots of *Pisum sativum*, and those of most other students

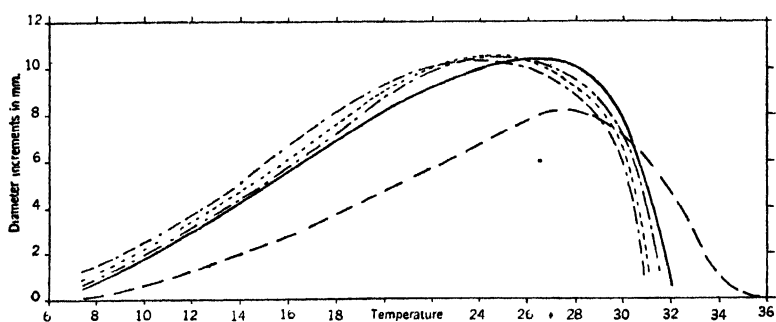


Fig. 2. Smoothed growth-temperature graphs for each of the first five 24-hour observation periods, for *Pythiacystis*.

First ————  
 Second ————  
 Third ————  
 Fourth ————  
 Fifth ————

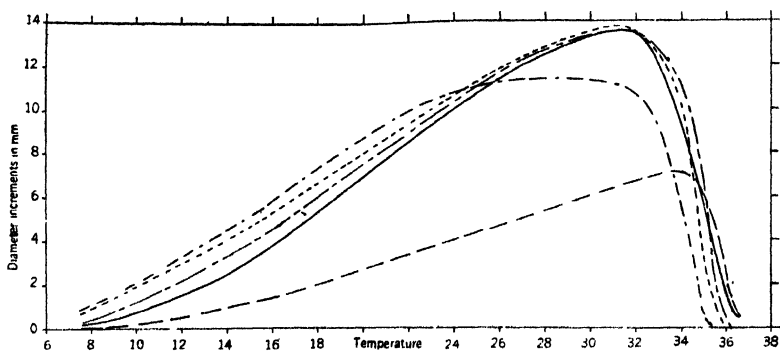


Fig. 3. Smoothed growth-temperature graphs for each of the first five 24-hour observation periods, for *Pythophthora*.

First ————  
 Second ————  
 Third ————  
 Fourth ————  
 Fifth ————

of life-process-temperature relations based on short time and temperature intervals. The graphs published by Brooks and Cooley (1917) showing the relations of growth of a number of apple rot fungi to temperature for 5-degree intervals also suggest the same general type of curve.

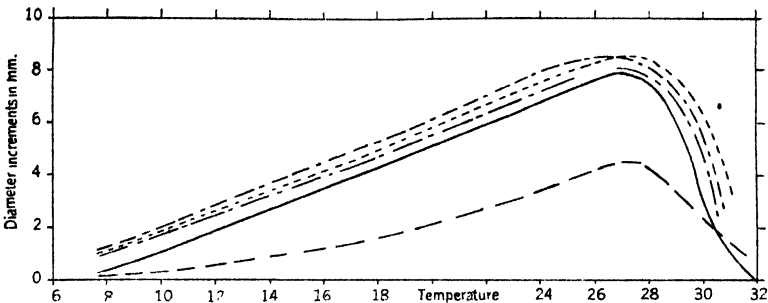


Fig. 4. Smoothed growth-temperature graphs for each of the first five 24-hour observation periods, for *Phomopsis*.

- First —  
Second —  
Third —  
Fourth - -  
Fifth —

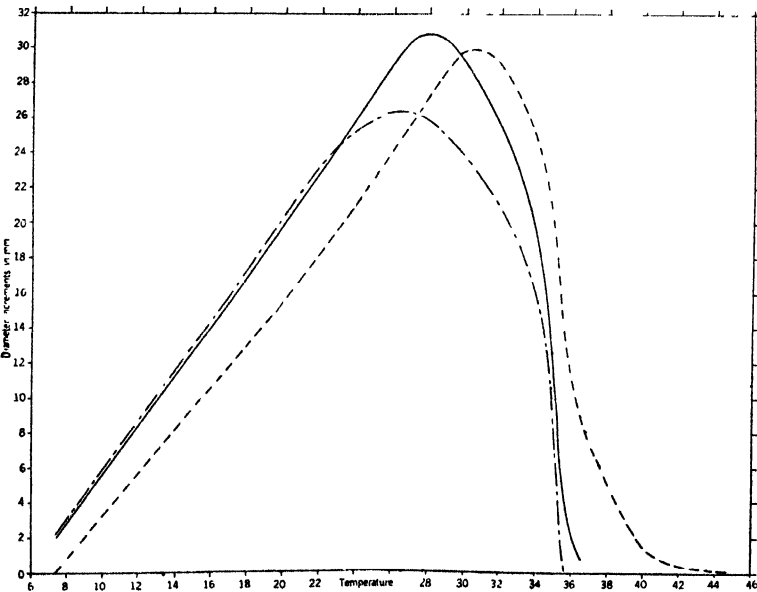


Fig. 5. Smoothed growth-temperature graphs for each of the first three 24-hour observation periods, for *Diplodia*.

- First —  
Second —  
Third —

## CHANGES IN THE GROWTH-TEMPERATURE RELATIONS

The four fungi all show very different growth-temperature graphs for the successive observation periods. For the same fungus the mean growth rate for any one of the successive 24-hour periods within the entire exposure period is generally not the same as that for any other 24-hour period. It follows from this that the growth-temperature graph for each organism alters its shape as we proceed from one observation period to another in the continuous series, as is clear from a superficial inspection of figures 2-5. This progressive change in the form of the growth-temperature graph, of course, represents a corresponding progressive change in the growth-temperature relation of the fungus, as time elapses after inoculation. Since the external conditions of these experiments are considered as not altering with time, this apparently gradual change in the growth-temperature relation must be evidence of internal physiological changes occurring in the organism.

When the curves for the successive 24-hour periods for each fungus (figs. 2-5) are compared certain general features can be noted. For every fungus there is a shifting of the apparent maximum temperature for growth downward (to the left in the graphs) with each successive observation period. This shifting is much more pronounced between the first and second 24-hour periods than between any other two consecutive periods, except in case of *Phytophthora*. For *Pythiacystis* the maximum shifts from about  $36^{\circ}$  for the first 24-hour period to about  $31^{\circ}$  for the fifth period; for *Phytophthora* the corresponding displacement is from about  $38^{\circ}$  to about  $35^{\circ}$ ; for *Diplodia* the maximum temperature changes from about  $46^{\circ}$  for the first 24-hour period to about  $35^{\circ}$  for the third period. The maximum temperatures for *Phomopsis* are more uncertain.

A similar displacement of the apparent temperature optimum (graph maximum) is shown for all the fungi excepting *Phomopsis*. The apparent optimum temperature for *Pythiacystis* shifts from about  $27.5^{\circ}$  for the first day to about  $24^{\circ}$  for the fifth day, the corresponding change for *Phytophthora* is from about  $34^{\circ}$  to about  $28^{\circ}$ , and for *Diplodia* the optimum is displaced from about  $31^{\circ}$  for the first day to about  $27^{\circ}$  for the third day.

Aside from the shifting of the apparent maximum and optimum temperature values just considered it should be noted that a similar

displacement is evident for growth rates lying within a large part of the suboptimal region of the growth-temperature graphs for each fungus. Throughout a large portion of this suboptimal region the ordinate value for any given maintained temperature is greater for every observation period after the first than it is for the immediately preceding period. This statement is true for *Pythiacystis* for the first five 24-hour periods after inoculation and for maintained temperatures up to 21° C. It is true for *Phytophthora* and *Phomopsis* for the first five observation periods for maintained temperatures up to 23.5° and 26° C. respectively. For *Diplodia* it is true for the first three 24-hour periods and for maintained temperatures up to about 21° C. In much of the supraoptimal region, on the other hand, the value of any given ordinate value is usually less than that for the next preceding period. The result of these shiftings in the specific relations of growth rate to maintained temperature is that the growth-temperature graph for each successive observation period intersects the next preceding graph. The only apparent exception to this statement is for two of the graphs for *Phomopsis*, for the third and fourth 24-hour periods. These relations to time are brought out more clearly in graphs of figures 8 and 9.

#### GROWTH-TEMPERATURE RELATIONS OF DIFFERENT ORGANISMS COMPARED BY MEANS OF GRAPHS OF EQUAL HEIGHT

To compare the curvatures of different graphs it is convenient to express all the ordinate values of each in terms of the maximum and to replot the graphs using the values thus derived. This treatment removes apparent differences in curvature due to differences in the magnitudes of the maximum ordinates. Such relative graphs, for the second 24-hour observation period for each fungus, are presented in figures 6 and 7. The upward and downward slopes of these four graphs are strictly comparable as to direction or angle, always with reference, not to actual growth rates in millimeters, but to relative growth rates, in terms of the corresponding maximum rate.

Referring to figures 6 and 7, the relative degrees of steepness of the four graphs are nearly the same for the suboptimal region and the same is true for the supraoptimal region, except that the graph for *Diplodia* is here somewhat less steep than are the other three. While the actual values are here hidden, the relative values as compared with the maximum growth may be compared for any process. The four graphs differ considerably in other details, however, mainly

in regard to total temperature range and in regard to minimum, optimum, and maximum temperature values.

Since all the graphs are brought to the same height by this treatment, the growth-temperature relations as a whole for one organism may be compared more directly with another irrespective of differences in actual growth increments. By this means the form of the growth-temperature graphs of a rapidly growing fungus, for example,

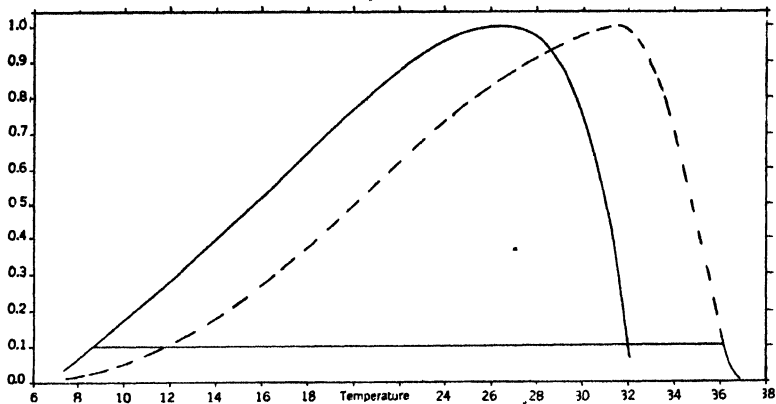


Fig. 6. Growth-temperature graphs for *Pythiacystis* and *Phytophthora* for the second 24-hour period after inoculation, the ordinate values being expressed in terms of the corresponding maximum growth rate taken as unity in each case.

*Pythiacystis citrophthora* —————  
*Phytophthora terrestris* - - - - -

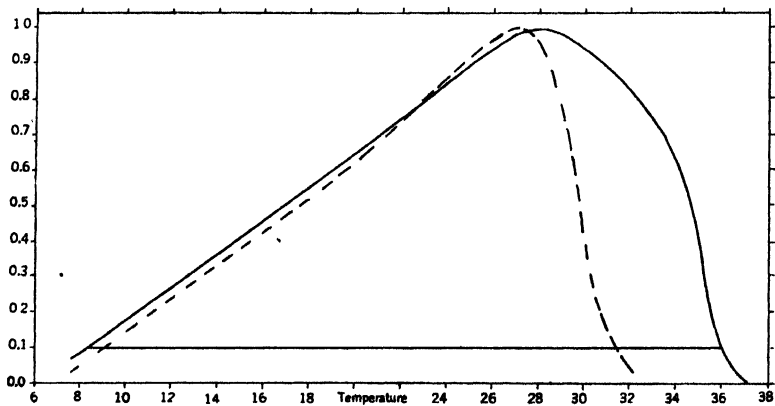


Fig. 7. Growth-temperature graphs for *Phomopsis* and *Diplodia* for the second 24-hour period after inoculation, the ordinate values being expressed in terms of the corresponding maximum growth rate as unity in each case.

*Diplodia natalensis* —————  
*Phomopsis citri* - - - - -

may be compared directly with those of a slow-growing one, or, furthermore, the rate of one kind of a process as influenced by temperature may be compared with the rate of any other process no matter how diverse or in what units each may be expressed.

It is to be noted that if the entire graph for *Phytophthora* were moved to the left through 4 degrees of temperature, e.g., if all the rates for this fungus were plotted at temperatures 4 degrees lower, this graph would follow closely the curve for *Pythiacystis*, except that the first part of the downward slope is a little steeper for *Phytophthora*. The difference between the two curves is, therefore, mainly one of location of the temperature range and the actual values of the increments. The extent of the temperature range, in number of degrees, and the values of the increments in relation to each other and to that for the optimum are nearly the same for these two fungi. The growth-temperature curves for the other two fungi, *Phomopsis* and *Diplodia*, show the optimum temperature close to the same point, 27° and 28° C. respectively. The maximum temperatures for these two, however, appear to be about 33° for *Phomopsis* and 37° for *Diplodia*, a difference of 4 degrees.

TABLE V

CHARACTERISTICS OF THE GRAPHS OF FIGURES 6 AND 7 FOR RATES EQUAL TO OR GREATER THAN 0.1 OF THE MAXIMAL RATE

Name of fungus	Extent of range in Deg. C.	Lower limit of range Deg. C.	Upper limit of range Deg. C.	Approximate optimum temp. Deg. C.	Per cent of range below optimum temp.
<i>Pythiacystis</i>	23.2	8.7	31.9	26.5	77.7
<i>Phytophthora</i>	24.1	12.0	36.1	31.5	80.5
<i>Phomopsis</i>	22.3	9.1	31.4	27.0	80.2
<i>Diplodia</i>	27.6	8.4	36.0	28.0	72.0

The total ranges of temperature can not be satisfactorily read from these graphs, although they are indicated in a general way. Since the interest of this discussion centers mainly about the forms of the graphs for the regions where the mean 24-hour rates are considerable, the range between the two temperatures giving a relative rate of one-tenth of the maximum rate may be considered instead of the total temperature range. This range is expressed as the length of the horizontal line lying between the two sides of the graph and having the constant ordinate 0.1. The graphs may be compared with respect to the magnitude of this partial range and also with respect to the relative position of the optimum temperature within this range. The main characteristics of the four graphs of figures 6 and 7 are shown in table V.



The extent of the temperature ranges for rates equal to or greater than 0.1 of the maximum rate for the four fungi are all considerably different; *Diplodia* has the greatest range ( $27.6^{\circ}$ ) and *Phomopsis* the smallest ( $22.3^{\circ}$ ). This partial temperature range has its lower limit lowest ( $8.4^{\circ}$ ) for *Diplodia*, a little higher for *Pythiacystis* ( $8.7^{\circ}$ ) and *Phomopsis* ( $9.1^{\circ}$ ), and highest for *Phytophthora* ( $12^{\circ}$ ). But the four fungi do not stand in this relation in regard to the upper limit of this range, for *Phytophthora* and *Diplodia* show about the same limit ( $36.1^{\circ}$  and  $36^{\circ}$ ), while *Pythiacystis* and *Phomopsis* also nearly agree in this respect ( $31^{\circ}$  and  $31.4^{\circ}$ ), the value for the last two being markedly lower than for the first two. Roughly speaking, it may be said that from about 70 to about 80 per cent of the temperature range here considered lies below the optimal temperature, with from about 30 to about 20 per cent lying above. Of course, such comparisons as these might be instituted between different fungi with reference to any other time period than the one here employed; only the mean rates of enlargement for the second 24-hour period after inoculation are here considered.

#### RELATION OF GROWTH RATE TO THE TIME OF EXPOSURE

It has been emphasized that the growth rates as measured in the work here reported differ not only for different fungi with the same maintained temperature and for different maintained temperatures with the same fungus, but also for different consecutive observation periods with the same fungus and the same maintained temperature, and it has also been pointed out that these last differences in growth rate must be regarded as due to progressive alterations in the internal conditions of the fungus as the culture becomes older.

This influence of time on rate of growth is best shown by the set of graphs shown in figures 8 and 9. Here the ordinates are in terms of diameter increase, but the abscissas represent successive 24-hour periods after exposure to a given temperature. Each graph shows growth on successive days at a given maintained temperature.

Inspection of tables I-IV and the graphs (figures 8 and 9) shows that the mean rate of enlargement alters with the age of the culture in three general ways. (1) For lower temperatures the rate increases throughout the culture period, the rate of increase being generally greatest for the first two days and much more gradual afterwards. (2) For a small range of higher temperatures the rate first increases

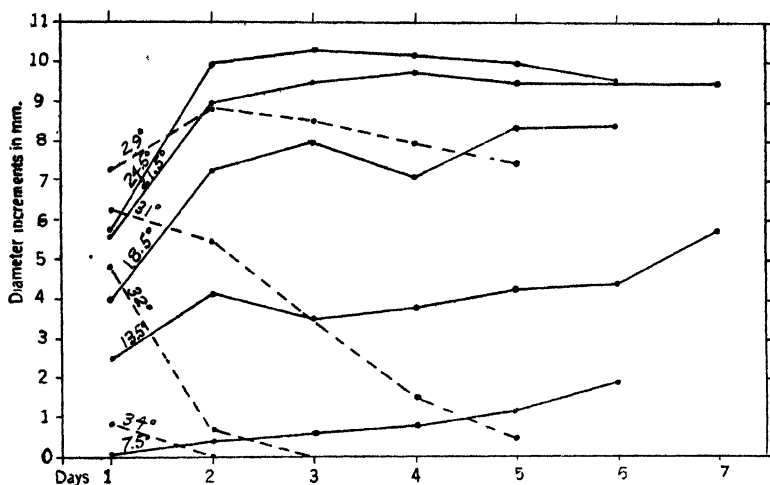


Fig. 8. Graphs showing relation of rate of enlargement to age of culture, for *Pythiarcystis* grown with various maintained temperatures. Ordinates are 24-hour increments and abscissas are number of days from moment of inoculation.

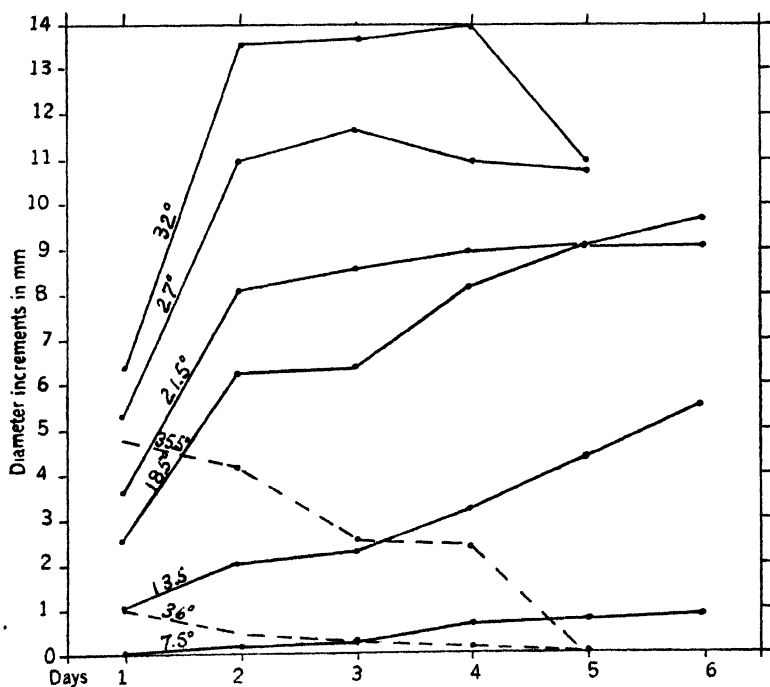


Fig. 9. Graphs showing relation of rate of enlargement to age of culture, for *Phytophthora* grown with various maintained temperatures.

and then remains constant or oscillates till the end of the culture period. (3) For the highest temperatures with which growth proceeds the rate decreases throughout the culture period, this decrease soon bringing the value of the growth rate to zero.

These graphs which indicate roughly the change of rate with time at various maintained temperatures suggest that we may have here a family of curves, which if the data were sufficient would be capable of mathematical treatment. It may be seen from tables I to IV and graphs of figures 8 and 9 that in general the rates increased with the time at temperatures below about 20° C. For example, with *Pythiacystis* the upper limit was about 19.5° C. and with *Phytophthora* it was about 21.5° for the 6 days tested. The growth rate in general decreased with time from the second day and thereafter at, and above, about 30° C. With *Pythiacystis* it was at or above 27.5° C. and with *Phytophthora* at or above 30° C. These same effects are apparent in some of the graphs showing the relation of temperature to the growth of apple rot fungi published by Brooks and Cooley (1917).

That the increase or decrease in growth rate with lapse of time in the present study was not due to the accumulation of products in the yet unused portions of the medium was shown, at least for *Pythiacystis*, by special tests in which fresh medium was placed at the advancing edges of parts of the mycelial disks after they had grown for two days. The subsequent rate of advance of the mycelial disk upon the fresh medium at the various maintained temperatures was the same as that upon the remainder of the unoccupied medium that had been in the dishes from the start.

## TEMPERATURE COEFFICIENTS

*Introductory.*—A temperature coefficient as here considered may be defined as the ratio of the rate of a given process at any given temperature to the rate at another temperature at a fixed interval below the first temperature. While the temperature interval considered may have any magnitude desired, it has been usual to consider in most chemical and physiological studies an interval of 10 degrees centigrade. In some investigations, where the rate considered alters greatly for small differences of maintained temperature, temperature coefficients for smaller intervals have been used.

The temperature coefficient for the rate of any process for a difference of 10 degrees is frequently represented by the symbol  $Q_{10}$ . When derived directly from experimental data showing rates for temperature intervals of 10 degrees this coefficient is, of course, the quotient obtained by dividing the rate for the higher temperature by the rate for the lower. It is, however, frequently calculated from data at irregular temperature intervals. The values obtained from such data by the employment of the usual formulae appear to be reliable only when the coefficient is constant, or nearly so, over a considerable range of temperature. Where the coefficient is changing rapidly with successive intervals of temperature, as is the case in many physiological processes, such derived coefficients are apt to be misleading.

Temperature coefficients for physiological processes have been much discussed in the literature. The statement occurs in numerous papers that the rate of a certain process under consideration does, or does not, obey the "Van't Hoff-Arrhenius rule" for chemical-reaction velocities with change in temperature, this rule being commonly understood to mean that the reaction velocity is continuously doubled or trebled for each rise of 10 degrees centigrade. It has been usual for some biologists and chemists to use this rule, as thus understood, to decide whether a given process should be regarded as chemical or physical in its nature. If the rate of the process in question be found to have a 10-degree temperature coefficient lying between 2 and 3, this is often considered an indication that the process dealt with is a chemical one or is controlled by chemical reactions. If, on the other hand, the 10-degree temperature coefficient proves to be much below 2,

this is taken as an indication of a physical reaction. In many discussions of temperature influence on process rates it has been assumed that if this coefficient appears to be more or less nearly constant for several 10-degree ranges and has a magnitude between 2 and 3 for the particular ranges studied, then the process follows the Van't Hoff-Arrhenius rule. If, on the other hand, the coefficient be not constant with higher and lower 10-degree ranges, but varies greatly above 3 or below 2, it is considered that the rule does not hold. This common, narrow interpretation of the Van't Hoff-Arrhenius rule appears to have been based on a general misconception of the Van't Hoff formula. It has been clearly pointed out by Stuart (1912) that Van't Hoff's formula itself makes clear that a constant coefficient is not implied even for chemical reactions and that Van't Hoff (1896) himself recognized that the coefficient values of different 10-degree ranges are by no means to be taken as constant; they are generally smaller with higher 10-degree ranges and larger with lower ones. All that Van't Hoff did was to make a very rough generalization and to point out that in many chemical reactions within the temperature usually dealt with in experimental observations it was interesting to note that the temperature coefficient was apt to fall between 2 and 3. If it is not usual for the temperature coefficient to be constant for simple chemical reactions, it is not to be expected that it would be constant for physiological processes, where much more complex reactions take place. An examination of the experimental data on the relation of a large number of life-processes to temperature shows that the temperature coefficients for such processes generally tend to diminish in value from lower to higher ranges of temperature.<sup>1</sup> Kanitz (1905) appears to have been one of the first to regard this feature as an essential in the analysis of the relationship between rates of life-processes and maintained temperatures.

Trautz and Volkmann (1908) gave considerable attention to this variation in the magnitudes of the temperature-coefficients for certain chemical processes, and Snyder (1911) pointed out that since it is the rule for the temperature coefficients of chemical reactions to vary with temperature such variation should be expected in physiological processes. Livingston (1916) noted that the temperature coefficient of the growth rates of maize seedlings, as determined by Lehenbauer (1914), might be regarded as following the Van't Hoff rule, as commonly understood, only for a very limited range of temperatures.

<sup>1</sup> Data for a large number of life-process rates, with citations of 363 papers, have been collected and compiled in a monograph by Kanitz (1915).

Leitch (1916), using short exposure periods, found that for the growth of *Pisum sativum* the temperature coefficient ( $Q_{10}$ ) decreased gradually from 8.25 for the interval between 0° and 10° C. to 2.2 between 18° and 28° C. The coefficients given by Lehenbauer (1914) for maize seedlings decrease from 6.56 for the 10-degree interval between 12° and 22° C. to 1.88 for the interval between 22° and 32° C. Rahn (1916), taking his data from experiments of Marshall Ward (1895) on the rate of development of *Bacillus ramosus* and *B. coli*, constructed some curves of the temperature coefficients, showing how these decrease rapidly from high values for low temperature intervals to low values for higher temperature intervals. Matthaei's (1905) data for the rate of carbon assimilation with temperature, from which Blackman derived the temperature coefficient of 2.1, show that the coefficient varied greatly even at the lower ranges, where the time factor was least operative, from a high value for the lowest intervals to a much lower value for higher intervals. Moore's (1918) work with the influence of temperature on the rate of heart beat of *Fundulus* embryo shows that the temperature coefficient decreases progressively from 7.6 at the temperature interval 2.5° to 12.5° C. to 1.4 for the interval 25° to 35° C. Denny (1916), reviewing the monograph by Kanitz (1915) on temperature and life processes, says:

"Many processes in living organisms show a temperature coefficient approximately that of the Van't Hoff law (2 to 3) within certain limits. Among the plant processes for which this has been found to be the case the following may be mentioned: CO<sub>2</sub> assimilation (Matthaei) between 0° and 37° C.; respiration of seedlings (Kuijper) between 0° and 35° C.; geotropic presentation time (Rutgers) between 5° and 25° C.; phototropic presentation time (M. S. De Vries) between 5° and 25° C.; protoplasmic streaming in *Elodea* (Velton) between 2.5° and 35° C.; permeability of plant cells and tissue (Rysselberghe) between 0° and 30° C.; intake of water by barley grains (Brown and Worley) between 3.8° and 34.6° C."

An examination of the data in most of these cases will show that while the coefficients are of the order of magnitude required by the so-called "Van't Hoff rule," in a majority of cases there is (even within the limited range to which the rule is supposed to apply) a marked tendency for them to decrease from lower to higher intervals of temperature; so that one may conclude that even these coefficients form part of a coefficient-temperature curve which if extended to the left would approach infinity and if extended to the right would arrive

at zero. The temperature coefficients given by Loeb and Chamberlain (1915) for the rate of segmentation of *Arbacia* decrease from 6 for a temperature interval between 8° and 18° C. to 2.5 between 15° and 25° C.

*Temperature Coefficients in the Present Study.*—The growth-temperature relations of the four fungi used in the work here reported were studied in certain aspects by means of such temperature coefficients as have just been considered. Since unexplained fluctuations in growth rate as related to temperature are to be neglected, it being desired to obtain information of a general nature only, the mean 24-hour rates for the various 24-hour observation periods (given in tables I-IV) were not employed in calculating the coefficients. Instead of

TABLE VI

MEAN 24-HOUR RATES OF ENLARGEMENT FOR CONSECUTIVE 1-DAY OBSERVATION PERIODS, FOR *PYTHIACYSTIS*, AS DETERMINED BY MEASURING THE ORDINATES OF THE SMOOTHED GRAPHS OF FIGURE 2

Temperature deg. C.	First day mm.	Second day mm.	Third day mm.	Fourth day mm.	Fifth day mm.
8	.3	.8	.9	1.1	1.4
9	.5	1.2	1.4	1.6	2.0
10	.7	1.7	1.9	2.2	2.4
11	1.0	2.3	2.5	2.8	3.1
12	1.3	2.9	3.0	3.4	3.7
13	1.6	3.5	3.6	4.0	4.2
14	2.0	4.1	4.3	4.7	5.0
15	2.4	4.8	5.0	5.4	5.8
16	2.8	5.5	5.7	6.2	6.5
17	3.3	6.2	6.5	6.8	7.2
18	3.7	6.8	7.1	7.5	8.0
19	4.2	7.4	8.0	8.3	8.7
20	4.7	8.0	8.7	8.9	9.2
21	5.1	8.6	9.3	9.5	9.7
22	5.7	9.1	9.8	9.9	10.0
23	6.1	9.6	10.2	10.3	10.2
24	6.6	9.9	10.4	10.5	10.3
25	7.1	10.2	10.4	10.4	10.1
26	7.6	10.4	10.3	10.2	9.9
27	8.0	10.4	10.1	9.8	9.5
28	8.1	10.2	9.6	9.3	8.8
29	7.8	9.4	8.7	8.3	7.9
30	7.1	7.8	7.4	6.9	6.4
31	6.1	5.3	3.5	1.5	.5
32	4.8	.7	0	0	0
33	2.6	0	-----	-----	-----
34	.9	-----	-----	-----	-----
35	.4	-----	-----	-----	-----
36	0	-----	-----	-----	-----

these, the length of the ordinate for each degree on each of the smoothed graphs of figures 2-5 were used. These ordinate values are presented in tables VI-IX. The arrangement and notation of the first parts of tables I-IV are here followed.

From these ordinate values of the smoothed growth-temperature graphs were calculated temperature coefficients for every 10-degree interval by whole degrees, between the lowest and highest maintained temperatures tested for each of the consecutive 24-hour observation periods represented in tables VI-IX. To illustrate the method followed, the mean 24-hour growth rate for *Pythiacystis* for the first day after inoculation is seen to be 0.3 mm. for a maintained temperature of 8° and 3.7 mm. for a maintained temperature of 18° C. The

TABLE VII

MEAN 24-HOUR RATES OF ENLARGEMENT FOR CONSECUTIVE 1-DAY OBSERVATION PERIODS, FOR PHYTOPHTHORA, AS DETERMINED BY MEASURING THE ORDINATES OF THE SMOOTHED GRAPHS OF FIGURE 3

Temperature deg. C.	First day mm.	Second day mm.	Third day mm.	Fourth day mm.	Fifth day mm.
8	.07	.25	.4	1.0	1.1
9	.15	.4	.9	1.5	1.6
10	.2	.7	1.2	2.0	2.1
11	.3	1.0	1.7	2.5	2.7
12	.5	1.4	2.2	3.0	3.3
13	.7	1.9	2.7	3.5	3.9
14	.9	2.4	3.3	4.1	4.5
15	1.1	3.0	3.9	4.7	5.2
16	1.4	3.7	4.5	5.3	5.8
17	1.7	4.4	5.3	5.9	6.5
18	2.0	5.2	6.0	6.6	7.2
19	2.4	6.0	6.7	7.2	7.9
20	2.7	6.9	7.4	7.9	8.6
21	3.0	7.8	8.2	8.7	9.3
22	3.3	8.6	9.0	9.3	9.9
23	3.6	9.3	9.8	10.1	10.3
24	4.0	10.0	10.5	10.7	10.7
25	4.3	10.8	11.1	11.3	10.9
26	4.6	11.4	11.8	11.9	11.1
27	5.0	11.9	12.3	12.4	11.3
28	5.3	12.5	12.8	12.9	11.3
29	5.7	12.9	13.2	13.3	11.3
30	6.0	13.3	13.4	13.6	11.2
31	6.4	13.5	13.6	13.7	11.0
32	6.7	13.5	13.6	13.6	10.7
33	6.9	12.2	12.8	12.6	9.2
34	7.1	9.5	11.2	10.2	6.0
35	6.1	5.7	6.8	4.2	1.0
36	2.2	1.7	.5	.2	.....



ratio 3.7 : 0.3 is 12.3, which is the 10-degree coefficient ( $Q_{10}$ ) for the 10-degree interval from  $8^{\circ}$  to  $18^{\circ}$  C. Now, since the value of  $Q_{10}$  varies with the maintained temperature, if its fluctuations are to be studied it is necessary to calculate the different values, not for successive 10-degree intervals (as  $8^{\circ}$ – $18^{\circ}$ ,  $18^{\circ}$ – $28^{\circ}$ ,  $28^{\circ}$ – $38^{\circ}$ ), but for 10-degree ranges beginning with each successive whole degree for which data are available (as  $8^{\circ}$ – $18^{\circ}$ ,  $9^{\circ}$ – $19^{\circ}$ ,  $10^{\circ}$ – $20^{\circ}$ , etc.). If the value just obtained for the range  $8^{\circ}$ – $18^{\circ}$  C. be written  $Q_{10} (8^{\circ}$ – $18^{\circ}) = 12.3$ , then referring to table VI we may write  $Q_{10} (9^{\circ}$ – $19^{\circ}) = 8.4$ ;  $Q_{10} (10^{\circ}$ – $20^{\circ}) = 6.7$ ;  $Q_{10} (11^{\circ}$ – $21^{\circ}) = 5.1$ , etc. For convenience of reference and for facility in plotting these temperature coefficients for various 10-degree temperature ranges as they are made to shift by single degrees, the middle point of each 10-degree range is taken to represent the range itself. Thus the coefficient value plotted at  $13^{\circ}$  stands for the 10-degree temperature coefficient for the range ( $8^{\circ}$ – $18^{\circ}$ ) whose middle point is  $13^{\circ}$  (figs. 10 and 11).

TABLE VIII

MEAN 24-HOUR RATES OF ENLARGEMENT FOR CONSECUTIVE 1-DAY OBSERVATION PERIODS, FOR PHOMOPSIS, AS DETERMINED BY MEASURING THE ORDINATES OF THE SMOOTHED GRAPHS OF FIGURE 4

Temperature deg. C.	First day mm.	Second day mm.	Third day mm.	Fourth day mm.	Fifth day mm.
8	.1	.4	1.1	1.2	1.3
9	.2	.7	1.4	1.5	1.6
10	.3	1.1	1.8	1.8	2.0
11	.4	1.4	2.1	2.1	2.4
12	.5	1.9	2.4	2.5	2.8
13	.6	2.2	2.8	2.9	3.2
14	.8	2.6	3.1	3.2	3.6
15	1.0	3.0	3.5	3.6	4.0
16	1.2	3.4	3.9	4.0	4.4
17	1.4	3.7	4.2	4.4	4.8
18	1.6	4.1	4.6	4.8	5.2
19	1.8	4.5	5.0	5.2	5.6
20	2.1	4.9	5.4	5.7	6.1
21	2.3	5.3	5.8	6.1	6.5
22	2.6	5.8	6.2	6.5	7.0
23	3.0	6.2	6.6	7.0	7.4
24	3.4	6.7	7.1	7.4	7.8
25	3.8	7.1	7.5	7.8	8.2
26	4.1	7.5	7.8	8.2	8.4
27	4.4	7.7	7.9	8.5	8.3
28	4.2	7.4	7.8	8.2	8.0
29	3.4	6.0	7.0	7.6	7.3
30	2.4	3.0	5.0	6.6	5.8
31	1.5	1.2	1.2	3.3	1.8
32	.9	.3	.2	-----	-----

The 10-degree coefficient values for all whole intervals of 10 degrees for which data are at hand for all four fungi and for each of the successive 24-hour observation periods employed in this experimentation are set forth in table X. The first column of this table presents the different temperature ranges, the second column shows the middle

TABLE IX

MEAN 24-HOUR RATES OF ENLARGEMENT FOR CONSECUTIVE 1-DAY OBSERVATION PERIODS, FOR DIPLODIA, AS DETERMINED BY MEASURING THE ORDINATES OF THE SMOOTHED GRAPHS OF FIGURE 5

Temperature deg. C.	First day mm.	Second day mm.	Third day mm.
8	.7	2.7	3.0
9	2.0	4.1	4.5
10	3.2	5.6	5.8
11	4.4	7.0	7.2
12	5.7	8.4	8.7
13	6.8	9.9	10.1
14	8.1	11.3	11.7
15	9.3	12.7	13.1
16	10.5	14.1	14.6
17	11.8	15.7	16.1
18	13.1	17.0	17.6
19	14.3	18.5	18.9
20	15.6	20.0	20.5
21	17.0	21.6	22.2
22	18.4	23.0	23.5
23	19.8	24.5	24.7
24	21.2	26.1	25.4
25	22.7	27.6	26.0
26	24.2	29.1	26.3
27	25.8	30.5	26.3
28	27.2	30.8	25.8
29	28.8	30.5	24.9
30	29.7	29.2	23.8
31	29.7	27.4	22.6
32	29.0	25.6	21.0
33	27.4	23.0	19.0
34	24.9	12.0	15.9
35	19.9	11.0	9.3
36	11.4	2.7	.....
37	7.4	.....	.....
38	5.0	.....	.....
39	3.0	.....	.....
40	1.5	.....	.....
41	.8	.....	.....
42	.5	.....	.....
43	.4	.....	.....
44	.3	.....	.....
45	.2	.....	.....



points of these ranges, which are to be taken as representing the various ranges themselves. The rest of the table falls into four parts, each part giving the data for a single fungus. Each single column gives the coefficients for a single one of the consecutive 24-hour observation periods.

Inspection of the coefficient values given in table X brings out the fact that, for every one of the four fungi and for each of the consecutive 24-hour observation periods, the 10-degree temperature coefficient for mycelial enlargement is greatest for the lowest temperature shown and regularly decreases toward higher temperatures, becoming smallest for the highest temperatures. The highest coefficient value here encountered (30) is that for 13° C. (range from 8° to 18°), for the first 24 hours after inoculation, for *Phytophthora*. This value is progressively smaller for progressively higher temperatures, becoming 0.47 for the temperature 31° (range from 26° to 36°). For the temperature 13° (range from 8° to 18°) the lowest coefficient value shown (4.0) is for the fourth 24-hour period for *Phomopsis*, and this value is progressively smaller for progressively higher temperatures, becoming 0.5 for the temperature 26° (range from 21° to 31°). The lowest coefficient value of the whole table is 0.01, for the temperature 31° (range from 26° to 36°) for the fourth 24-hour period for *Phytophthora*, this value being progressively larger with progressively lower temperatures and becoming 6.6 for the temperature 13°. (range from 8° to 18°).\*

Aside from the regular falling off in the coefficient value for each observation period and fungus, as we pass from lower to higher temperatures, as just pointed out, the value for any temperature and fungus is always largest for the first 24-hour period after inoculation and generally tends to become smaller with each successive period after the first, although this last statement is not always strictly true for all temperature ranges. The relation of the value of this temperature coefficient to the maintained temperature representing the middle point of the 10-degree temperature range from which each coefficient value is derived is shown graphically for the second 24-hour period after inoculation, for three of the fungi in figure 10. Abscissas represent these middle points, while ordinates are the corresponding coefficient values. These graphs have not been smoothed.

These three graphs of 10-degree temperature coefficients are seen to be alike in their general form. Every one begins with a relatively very high value at the left (lowest temperature range tested) and

descends, rapidly at first and then less rapidly, with higher temperatures.

From the nature of the temperature coefficient it is clear that its value for *any* range of maintained temperatures having its lower limit just below the *minimum temperature for enlargement* must be infinite, since the ratio of any positive quantity to zero is, of course, infinity.

On the other hand, as the temperature range for which the coefficient value is calculated has its upper limit approaching the maximum temperature for enlargement the coefficient value approaches zero. No matter what range of temperature is employed, a change of

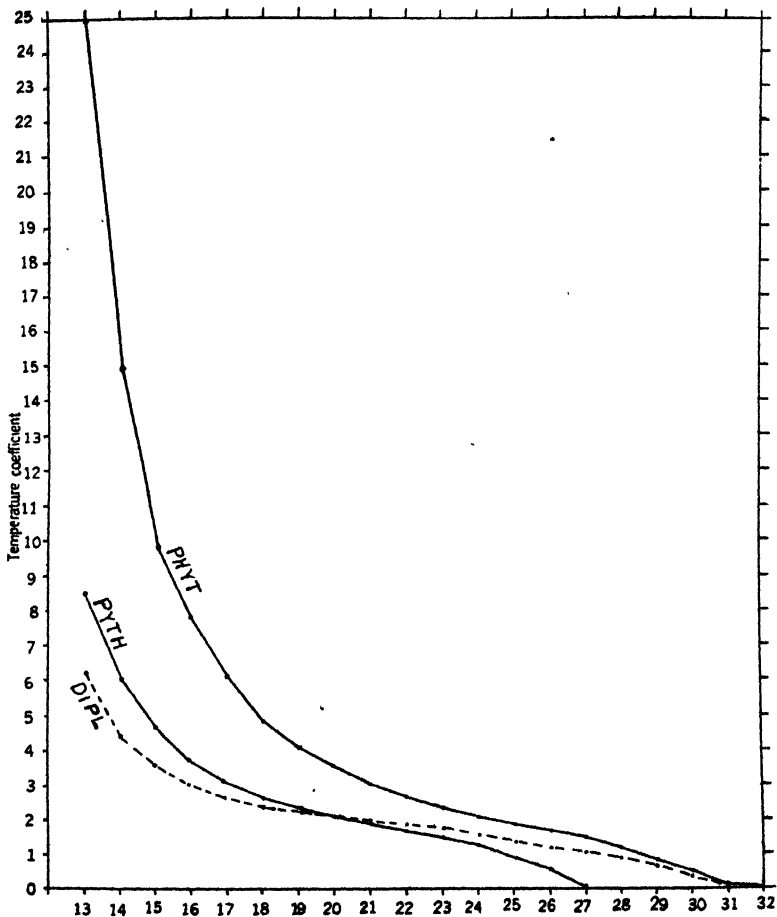


Fig. 10. Graphs of the 10-degree temperature coefficient, as related to temperature, for *Phytophthora*, *Pythiacystis* and *Diplodia*, for the second 24-hour period after inoculation.

maintained temperature from some value below the maximum temperature to some value above that cardinal point must be accompanied by a corresponding change in the rate of enlargement from a positive value to zero, and the ratio of zero to any positive quantity is, of course, zero.

For graphs such as those here considered it follows (from the points brought out above) that the slope of the graph at the point of maximum ordinate value (left end) appears to furnish a criterion by which it may be judged, at least in a general way, how nearly the abscissa of this point approaches the minimum temperature. An inspection of the curves at the lowest temperature range here considered ( $8^{\circ}$ – $18^{\circ}$  C.) indicates that its lower limit ( $8^{\circ}$  C.) is much more nearly the minimum temperature for enlargement for *Phytophthora* than it is for the other fungi. The curves also indicate that  $8^{\circ}$  is nearer the temperature minimum for *Pythiacystis* than for *Diplodia*.

Since the graph of temperature coefficient as related to temperature shows ordinates that decrease in magnitude from infinity to zero, it follows that there must be some point on every such graph at which the ordinate value is unity. This point at which the temperature coefficient value is unity will represent the middle point of a range within which lies the optimum temperature. The temperature value corresponding to this abscissa is, therefore, near the *temperature optimum* for the process considered. For lower temperatures the coefficient values are all greater than unity, for higher ones they are all smaller than unity.

A point that needs emphasis in studying the general nature of the temperature coefficients of most processes showing temperature minima and maxima is this, that every such process must show a certain temperature range for which the temperature coefficient has values between 2.0 and 3.0, etc. It is, therefore, quite without any definite meaning to state that the temperature coefficient for any process has a certain value, unless the corresponding temperature range is simultaneously stated. The coefficient value may be everything between zero and infinity, depending on the temperature range considered and upon the position of that range within the total temperature range of the process. The so-called Van't Hoff rule, stating that the temperature coefficients of many chemical reaction velocities have values between 2.0 and 3.0 is obviously true, therefore, if the proper temperature ranges are considered. It appears to be true that many simple chemical reactions and many physiological processes show temperature

coefficient values between 2.0 and 3.0 for certain temperature ranges within the ordinary range of weather temperatures on the earth, and it is perhaps this fact that has led to so much inadequate discussion about these coefficients, especially in physiological literature. Great emphasis should be placed upon the fact that the temperature coefficient for most processes having temperature limits is a continuously varying value, the variation proceeding from infinity to zero.

From this point of view the temperature relations of different processes under stated non-temperature conditions and with stated exposure periods are clearly comparable, not by means of single temperature coefficient values, but by means of the coefficient-temperature relation as a whole. Practically, the simplest way to present this relation for a given process is to construct such coefficient-temperature graphs as those shown in figures 10 and 11. The form and position of these graphs completely describe the rate-temperature relation. If two processes are to be compared in respect to this temperature relation, the comparison should be instituted between the two coefficient-temperature graphs, constructed on the same scale. If the two graphs coincide throughout, then the temperature relations of the two processes are alike; they have approximately the same temperature minima, optima, and maxima, and the two rates change from one temperature to another in just the same way. If the two graphs fail to coincide throughout, the two rate-temperature relations differ, and just how they differ is apparent from an inspection of the graphs. Furthermore, the different values of the temperature coefficient for the same process, etc., may readily be compared for different temperatures and the coefficient values for different processes may be compared for the same temperatures. Some of the points brought out by inspection of the group of three coefficient-temperature graphs shown in figure 10 have been mentioned, but many others not here considered may be noted.

The three graphs thus far dealt with show the relation of temperature coefficients to temperature for three of the fungi employed in this study and for the second 24-hour period after inoculation. The four coefficient graphs for *Pythiacystis*, for the first, second, third, and fourth 24-hour periods after inoculation, are shown in figure 11. These graphs are constructed from the data given in table X in the manner employed for figure 10; they have not been smoothed. The graph for each successive period after the first lies below the one for the preceding period. The progressive lowering (already mentioned)

of minimum, maximum, and optimum temperatures with the successive periods is clearly shown; also the difference between the growth-temperature relation for the first period and that for the second is shown to be far more pronounced than all the differences between these relations for successive periods.

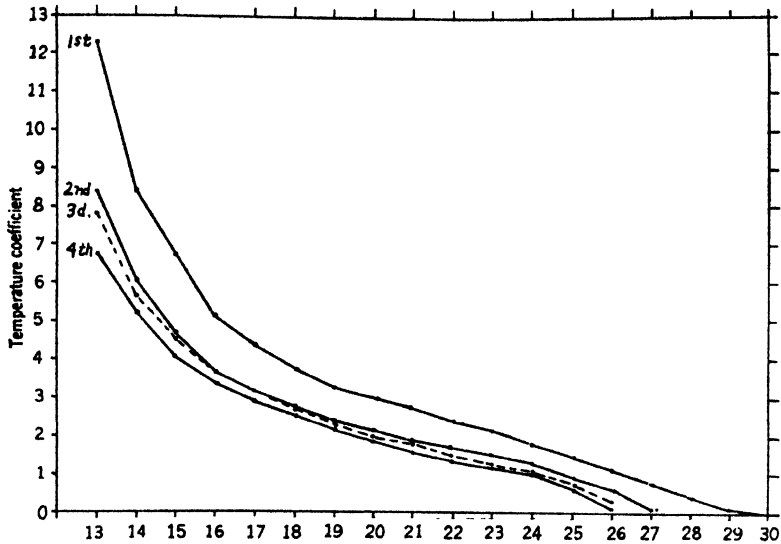


Fig. 11. Graphs of the 10-degree temperature coefficient, as related to temperature, for *Pythiacystis*, for four consecutive 24-hour observation periods within the 4-day exposure period.

## CONCLUSION

From the results of the investigation of the temperature relations of growth in pure cultures of four fungi (*Pythiacystis citrophthora*, *Phytophthora terrestria*, *Phomopsis citri*, and *Diplodia natalensis*), discussed in detail in the preceding pages, the following generalizations may now be brought together.

It was indicated that there is the usual optimum temperature above and below which the rate of enlargement was smaller with higher or lower maintained temperatures. Growth-temperature graphs (with temperatures as abscissas and growth rates as ordinates) rise from left to right (from lower to higher temperatures), being at first slightly concave upward, then becoming convex till the optimum is passed, and then falling rapidly toward the temperature axis.

The fact is to be emphasized that the optimum temperature for the average rate of growth of a given fungus with a given medium is



not always the same for different lengths of observation periods, or when periods of equal length have different time relations to the beginning of the culture period.

With culture periods of from three to six days and an observation period of 24 hours in length, it was found that in general the optimum temperature for growth shifted to lower temperatures for each successive observation period. There was also corresponding displacement of the apparent maximum temperature downward (from higher to lower temperatures) with each successive observation period.

A comparative study of the growth-temperature graphs of the four fungi for the second 24-hour period shows that the total range of temperature within which growth rate values are one-tenth or more of the maximum rate includes from 32.5 to 37 centigrade degrees of the temperature scale. Of this range, from 70 to 80 per cent is below the optimum temperature for growth.

With comparatively low temperatures the growth rate increases with the age of the culture throughout the culture period and with the highest temperatures it decreases throughout the culture period, this decrease soon bringing the value to zero. With a small range of intermediate temperatures, the rate first increases with time and then remains constant, oscillates or decreases.

The 10-degree temperature coefficient ( $Q_{10}$ ) for each of the four fungi has a high value at the lowest range studied and decreases progressively through lower values to zero. The form of the graphs representing the value of the temperature coefficient as related to different ranges of maintained temperature shows that the value of the temperature coefficient must begin with infinity for some low range, must pass through all finite values and then must reach zero for some higher range. For growth-temperature relations of this type the range for which the coefficient is unity will include the optimum temperature, the range for which the coefficient is infinity will include the minimum temperature, and the range for which the coefficient is zero will include the maximum temperature.

The use of the coefficient-temperature graphs furnishes a direct method of comparing the growth-temperature relations of different organisms, no matter in what units the rates have been expressed. If the graphs of two different processes coincide throughout, the growth-temperature relations must be considered to be the same. On the other hand, if the two graphs fail to coincide throughout, their lack of coincidence furnishes evidence of the particular manner in which the temperature relations of the two processes differ.

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# THE ALINEMENT CHART METHOD OF PREPARING TREE VOLUME TABLES

BY

DONALD BRUCE

UNIVERSITY OF CALIFORNIA PRESS  
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THE ALINEMENT CHART METHOD OF  
PREPARING TREE VOLUME TABLES<sup>1</sup>

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DONALD BRUCE

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The chief use of the alinement chart<sup>2</sup> is to express with the simplest possible system of lines a law the equation of which is known. The underlying principle is so flexible that almost any formula can be expressed thereby, although the most striking advantages over a system of rectangular coördinates do not appear unless three or more variables are involved. The axes may be parallel or converging, straight or curved, and graduated either uniformly or with intervals which vary in accordance with some given law, the form of the graph depending on the form of the equation. It follows from this very flexibility that such charts are, in general, unsuited for use with empirical data. The following pages, however, describe an exception to this general rule in which one type of alinement chart may be advantageously used in the preparation of tree volume tables, although the form of the equation of such a table is yet unknown.

The most suitable type of chart can be determined by working out an approximate algebraic expression for the volume of a tree in terms of its diameter and height. This expression is complicated by the fact that, for almost all American tables, volumes must be computed in board feet as scaled by some log rule, instead of in cubic feet. The starting point must therefore be the equation of the volume of a

<sup>1</sup> Acknowledgment is made to Professor Frank Irwin, of the Department of Mathematics of this University, for assistance in connection with the analytic features of this study.

<sup>2</sup> A complete discussion of the theory of alinement charts may be found in such works as: J. Lipka, *Graphical and Mechanical Computation*; J. B. Peddle, *The Construction of Graphic Charts*; and M. d'Ocagne, *Traité de Nomographie*. For a discussion of the application of certain simple types to some formulae of forest mensuration see D. Bruce, "Alinement Charts in Forest Mensuration," *Journal of Forestry*, XVII, 7, 773 (November, 1919).

log in board feet first formulated by Professor Daniels,<sup>3</sup> i.e.,  $v = ad^2 + bd + c$  (I). For simplicity, let us apply this formula to a tree of uniform taper up to its merchantable top limit, that is, one which is a frustum of a cone.

Let  $V$  = volume of a tree in feet b. m.

Let  $v$  = volume of a log in feet b. m.

Let  $D$  = d. i. b. stump (assumed equivalent to d. b. h.).

Let  $d$  = top diameter of a log.

Let  $H$  = height in logs of tree.

Let  $t$  = top d. i. b. of tree.

It is evident that the taper of the tree  $= D - t$ , and that the taper per log  $= \frac{D-t}{H}$ . Therefore the top diameters of the several logs of the trees are the terms of the following series:  $t, t + \frac{D-t}{H}, t + \frac{2(D-t)}{H}, t + \frac{3(D-t)}{H}, \dots$  to  $H$  terms, and the volumes of the same are the terms of the following series, each top diameter being successively substituted in I:

$$at^2 + bt + c;$$

$$at^2 + \frac{2at}{H}(D-t) + \frac{a}{H^2}(D-t)^2 + bt + \frac{b}{H}(D-t) + c;$$

$$at^2 + \frac{4at}{H}(D-t) + \frac{4a}{H^2}(D-t)^2 + bt + \frac{2b}{H}(D-t) + c;$$

$$at^2 + \frac{6at}{H}(D-t) + \frac{9a}{H^2}(D-t)^2 + bt + \frac{3b}{H}(D-t) + c;$$

$$at^2 + \frac{8at}{H}(D-t) + \frac{16a}{H^2}(D-t)^2 + bt + \frac{4b}{H}(D-t) + c;$$

$\dots$  to  $H$  terms.

$V$  = the sum of this series to  $H$  terms. This may be obtained by the differential method in which a new series of first differences is derived by subtracting each term from that which follows it, and this process is repeated, successively obtaining a series of second differences, third differences, etc., until all terms become zero and the series vanishes.

<sup>3</sup> See A. L. Daniels, *Measurement of Sawlogs*, Vermont Agr. Exp. Sta., Bulletin 102, 1903.

The sum then equals  $na + \frac{n(n-1)}{2} d_1 + n \frac{(n-1)(n-2)}{3} d_2 + \dots$  where  $n$  = number of terms,  $a$  = the first term of the original series,  $d_1$  = the first term of series of first differences,  $d_2$  = the first term of series of second differences, etc.

Series of first differences is:

$$\frac{2at}{H} (D - t) + \frac{a}{H^2} (D - t)^2 + \frac{b}{H} (D - t);$$

$$\frac{2at}{H} (D - t) + \frac{3a}{H^2} (D - t)^2 + \frac{b}{H} (D - t);$$

$$\frac{2at}{H} (D - t) + \frac{5a}{H^2} (D - t)^2 + \frac{b}{H} (D - t);$$

$$\frac{2at}{H} (D - t) + \frac{7a}{H^2} (D - t)^2 + \frac{b}{H} (D - t); \text{ etc.}$$

Series of second differences is:

$$\frac{2a(D-t)^2}{H^2}; \quad \frac{2a(D-t)^2}{H^2}; \quad \frac{2a(D-t)^2}{H^2}; \text{ etc.}$$

Series of the third differences is:

$$0; \quad 0; \quad 0; \text{ etc.}$$

$$\begin{aligned} \text{And } V = \text{sum of this series} &= H(at^2 + bt + c) + \frac{H(H-1)}{2} \\ &\left\{ \frac{2at}{H} (D - t) + \frac{a}{H^2} (D - t)^2 + \frac{b}{H} (D - t) \right\} + \frac{H(H-1)(H-2)}{6} \\ &\left\{ \frac{2a}{H^2} (D - t)^2 \right\} \end{aligned}$$

Expanding and rearranging in terms of  $D$ , this becomes:

$$\begin{aligned} V &= \left\{ \frac{a(H-1)(2H-1)}{6H} \right\} D^2 + \frac{(H-1)}{6H} \left\{ (2at + 3b)H + 2at \right\} D \\ &+ \left\{ \frac{(2at^2 + 3bt + 6c)H}{6} + \frac{(3at^2 + 3bt)}{6} + \frac{at^2}{6H} \right\} \quad (\text{II}) \end{aligned}$$



Rearranging in terms of  $H$ , this may also be written :

$$V = \frac{H}{6} \left\{ 2aD^2 + (2at + 3b) D + 2at^2 + 3bt + 6c \right\} - \frac{1}{2} \left\{ aD^2 + bD - t(at + b) \right\} + \frac{a}{6H} (D^2 - 2tD + t^2) \quad (\text{III})$$

Let us now apply this general formula to a specific case, for example, that of trees scaled by the Scribner log rule to a six-inch top cutting limit. A close approximation formula for this log rule (for 16-foot lengths) is:

$$V = .765d^2 - .55d - 21.$$

We therefore may assume

$$a = .765$$

$$b = -.55$$

$$c = -21$$

$$t = 6.$$

Substituting these values in III, we have :

$$V = H (.255D^2 + 1.255D - 13.47) - (.3825D^2 - .275D - 12.12) + \frac{1}{H} (.1275D^2 - 1.53D + 4.59). \quad (\text{IV})$$

Typical equations for the height class curves of a volume table in graphic form can now be found by substituting in IV given values of  $H$ ; for example:

$$\text{For } H = 2, V = .19125D^2 + 2.02D - 12.52 \quad (\text{V})$$

$$H = 6, V = 1.16875D^2 + 7.55D - 67.93 \quad (\text{VI})$$

$$H = 10, V = 2.18025D^2 + 12.672D - 122.121 \quad (\text{VII})$$

Similarly, typical diameter class curves are:

$$\text{For } D = 10, V = 24.58H - 23.38 + \frac{2.04}{H} \quad (\text{VIII})$$

$$D = 20, V = 113.63H - 135.4 + \frac{25.99}{H} \quad (\text{IX})$$

$$D = 30, V = 253.68H - 323.88 + \frac{73.44}{H} \quad (\text{X})$$

$$D = 50, V = 686.78H - 930.38 + \frac{246.84}{H} \quad (\text{XI})$$

It will readably be seen that V, VI, and VII are equations of parabolas, while VIII and IX and X and XI are hyperbolas. These deductions agree so well with the actual results obtained in volume tables constructed by the conventional method on a similar basis that it seems probable that the general form of the equation should apply at least approximately to actual trees as well as to the cone frusta on which it is based. Furthermore, it has been tentatively established, and without any conflicting evidence coming to light, that, in the case at least where a fixed top cutting limit is used, frustum form factors are functions of diameter and not of height. If this is true, such equations as VIII can be corrected to apply accurately to any given species by multiplying into them the proper form factors, which would merely change the values of the constants without affecting the form. Finally, the ease with which the alinement chart devised to apply to cone frusta works out for actual trees is the best proof of the adequacy of the equation.

Next, it is necessary to determine this alinement form. Unfortunately a difficulty at once presents itself. The equation appears to be one of those rare instances which cannot be thus expressed.<sup>4</sup> It has been found by experiment, however, that if two parallel axes be assigned to  $V$  and  $H$ , the former graduated uniformly upward and the latter uniformly downward, all lines expressing a single value of  $D$  (taken from a table of values of volumes of cone frusta in board feet or calculated by the above formulae) will intersect nearly (but not quite) in a common point, and that these common points for a series of values of  $D$  lie almost (but not quite) in a straight line, which if produced will pass through the zero point on the  $V$  axis. Figure 1 illustrates this fact, although a larger scale is needed to bring out the failure of the lines to intersect perfectly.

The reason for this becomes evident upon analysis. Let the lower left-hand corner of figure 1 serve also as the origin of a system of rectangular coördinates with one unit equaling ten of the small squares. Also let  $b$  equal the width of the paper. Then any straight line used in solving values by the alinement chart can be expressed

<sup>4</sup> Only those equations can be expressed by an alinement chart that can be put in the determinant form

$$\begin{vmatrix} f_1 & g_1 & h_1 \\ f_2 & g_2 & h_2 \\ f_3 & g_3 & h_3 \end{vmatrix} = 0$$

where  $f_i$ ,  $g_i$ , and  $h_i$  are functions of  $x$ , ( $i = 1, 2, 3$ ).

as connecting the points  $(0, 22 - 2H)$  and  $\left\{b, \frac{V}{100}\right\}$  or by the equation

$$Y = \left\{ \frac{V}{100} - 22 + 2H \right\} \frac{X}{b} + 22 - 2H. \quad (\text{XI})$$

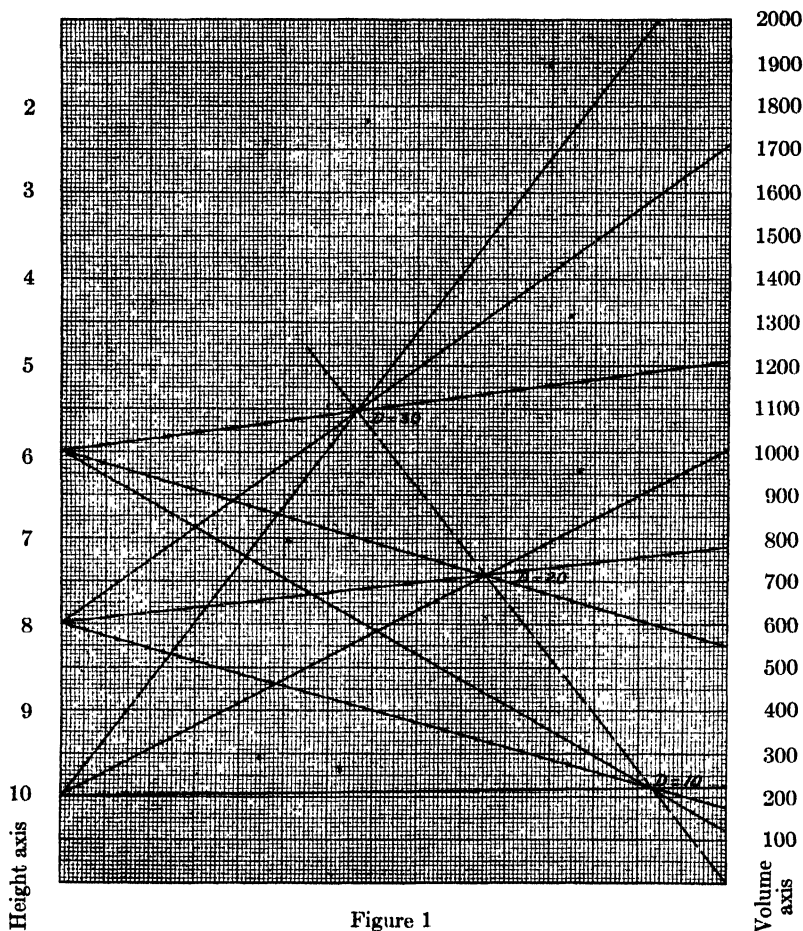


Figure 1

Alinement Form giving approximately correct results for volumes of cone frusta in feet b.m.

Now from equation IV,  $V = AH + B + \frac{C}{H}$  (where  $A, B, C$  are functions of  $D$ ) and the equations of two such lines corresponding to any values of  $H$ , such as  $H_1$  and  $H_2$ , and having a common value of  $D$ , will then be (from equation XI):

$$Y = \left\{ \frac{AH_1 + B + \frac{C}{H_1}}{100} - 22 + 2H_1 \right\} \frac{X}{b} + 22 - 2H_1$$

and

$$Y = \left\{ \frac{AH_2 + B + \frac{C}{H_2}}{100} - 22 + 2H_2 \right\} \frac{X}{b} + 22 - 2H_2$$

The point of intersection of these two lines can now be found by solving these two equations simultaneously, and when this is done the following values of  $X$  and  $Y$  are obtained:

$$X = \frac{200b}{A - \frac{C}{H_1 H_2} + 200} \quad (\text{XIII})$$

$$Y = \frac{22 \left\{ A - \frac{C}{H_1 H_2} \right\} + 2B + \frac{2C}{H_1} + \frac{2C}{H_2}}{A - \frac{C}{H_1 H_2} + 200} \quad (\text{XIV})$$

It will be seen that, for such a range of values for  $D$  and  $H$  as are actually encountered, these two equations approximate quite closely to

$$X = \frac{200b}{A + 200} \quad (\text{XV})$$

$$Y = \frac{22A + 2B}{A + 200} \quad (\text{XVI})$$

Since both  $X$  and  $Y$  in this last pair of equations are independent of  $H$ , this approximation explains the approximate common intersection of all lines having a given value of  $D$ . They can, moreover, be combined to give an equation of the curve upon which all these common intersections fall, but the result is in a form too complicated to be of much value, although it can be readily identified as an equation of a conic section (obviously a very straight portion of a hyperbola). The curve can be plotted more simply by means of equations XV and XVI, and will be found to be very nearly a straight line passing through the zero point on the  $V$  axis.

It seems as if a regraduation of the  $H$  axis might be made to result in perfect instead of approximate intersections. It will be found that this can be readily accomplished for any given value of  $D$  on the assumption that the  $H$  graduating distance from a fixed point  $= KH$

$+ L + \frac{M}{H}$  where  $K$ ,  $L$ , and  $M$  are constants. But unfortunately these three constants prove to be themselves functions of  $D$ . In other words, different sets of graduations would have to be used for each value of the diameter, which is obviously impracticable.

Each value of  $H$ , however, is in practice associated with a rather narrow range of  $D$  values. It is therefore possible to modify the positions of the  $H$  graduations empirically so as to result in a decided improvement in the intersections, and at the same time a slight readjustment of the  $D$  axis can be made with advantage. The best results appear to be obtained by the following plan:

1. Graduate the  $V$  axis as already described.
2. Graduate the  $H$  axis as already described but omit all values under that of 5 logs.
3. Select a few definite values of  $D$  well distributed over the desired range, and for each calculate the volumes for three or more values of  $H$  ( $H=5$  or over). If a table of cone frusta is available, these volumes may, of course, be taken therefrom.
4. Draw straight lines from each value of  $H$  to the corresponding value of  $V$ , and select points which appear to be the averages of the intersections of lines relating to each common value of  $D$ .
5. Draw a curve through the points thus selected. For most work a sufficiently close approximation will be found to be a straight line passing through the  $V$  origin.
6. Enter on this curve or straight line the  $D$  graduations indicated by the straight lines of step 4.
7. Obtain two or three values for  $V$  corresponding to the lower values of  $H$  (under 5 logs) and to the values of  $D$  already graduated. By drawing the appropriate straight lines their intersections with the  $H$  axis will indicate the best average positions of the smaller  $H$  graduations. These should then be entered on that axis.
8. Complete the graduation of the  $D$  axis by intersection, using for each value of  $D$  appropriate values of  $H$ .

The following table, worked out by the above process, may also be used directly to save time and labor.

TABLE I  
GRADUATION OF *H* AXIS

Height in logs Intersection with the diagonal axis	Distance from fixed point on axis	
	Values to be used where the heights are in logs and tenths of logs	Values to be used where the heights are in feet
	1.26	20.16
2	2.08	33.28
3	3.03	48.48
4	4.01	64.16
5	5	80
6	6	96
7	7	112
8	8	128
9	9	144
10	10	160
11	11	176

In the application of this theory to the making of a volume table the successive steps may be as follows:

1. Prepare an incomplete alinement graph (fig. 2) such as has been illustrated in Figure 1, but with the *H* axis graduated in accordance with the table just given.

2. Draw a diagonal straight line representing the *D* axis between the point representing its intersection with the *H* axis and the zero point on the *V* axis.

3. Graduate the *D* axis as follows: Each tree measurement (a sufficient number of which are supposed to be at hand) is used to draw a straight line between the point on the *H* axis corresponding to the height of the tree and the point on the *V* axis corresponding to the volume of the tree. The intersection of this with the *D* axis is an indication of the position of the *D* graduation corresponding to its diameter. The indications of a number of trees will naturally be more or less conflicting and the results must therefore be evened off by a graduating curve such as indicated in figure 3. In this curve the distance of each intersection above the base of the graph of figure 2 is plotted over its corresponding diameter. When all the points are thus plotted a smooth curve is drawn through them, and from this curve the *D* axis is finally graduated.

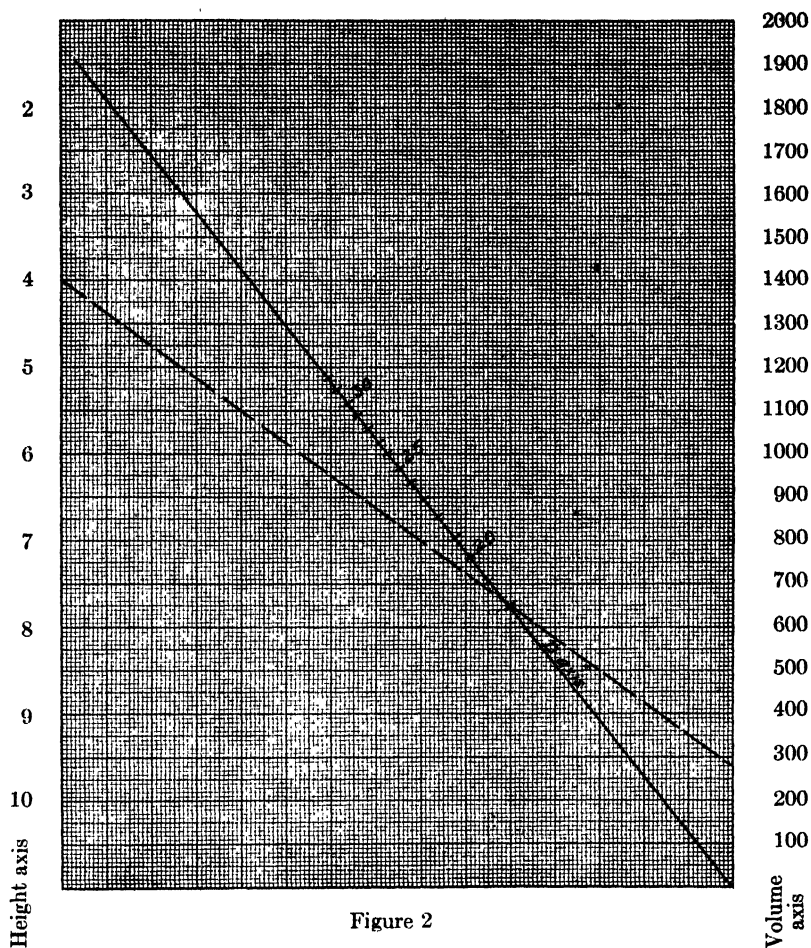


Figure 2

An alignment volume table.

Table II gives the basic tree data used in this illustrative instance. The broken line in figure 2 shows how the first values of this table are plotted. The left-hand point on figure 3 is plotted from the resulting intersection with the *D* axis.

It is immaterial whether each tree is thus used to determine a point on the graduating curve or whether the average volume, height, and diameter of the height-diameter classes are used. In the latter case, each point should, of course, be weighted in accordance with the number of trees which are thus averaged together. Figure 3 was drawn in accordance with the latter method.

4. The volume table can now be read from the completed alignment chart, the result being given in Table III.

TABLE II  
BASIC TREE DATA, WHITE FIR, STANISLAUS NATIONAL FOREST  
(Measurements taken by U. S. Forest Service)

No. of trees in class	Average D. B. H. inches	Average merchantable height in 16-ft. logs	Average Vol. ft. B. M.
7	18.1	4.0	280
3	19.5	3.9	330
8	19.9	4.0	340
5	19.8	5.0	500
6	21.9	4.4	540
7	22.2	5.1	640
6	21.8	6.0	670
1	22.8	6.5	810
2	23.9	4.1	510
1	23.4	4.2	490
9	23.7	5.2	640
6	24.1	6.2	970
2	24.6	7.3	1230
4	25.5	5.0	660
9	25.9	5.6	950
12	26.3	6.0	970
9	26.2	7.0	1270
1	25.8	7.6	1510
4	27.7	5.6	1050
10	28.3	6.3	1250
7	28.1	7.5	1450
1	27.6	8.3	1760
1	29.2	4.6	1090
7	29.9	6.0	1300
7	29.8	6.4	1280
12	29.7	7.3	1640
3	30.2	7.9	1720
16	31.6	6.6	1600

TABLE III  
VOLUME TABLE READ FROM FIGURE 2

D. B. H.	Height in 16-ft. logs				
	4	5	6	7	8
18	280	380			
20	350	480			
22	430	590	740	900	
24	510	690	880	1060	
26		790	1000	1220	1430
28		850	1130	1370	1600
30		1010	1280	1550	1820
32		1140	1450	1750	2060



In most cases it will be found that the graduating curve of figure 3 can be entered on the same sheet as the alinement chart without confusion and with a saving in time and convenience. In step 3, moreover, it is not necessary actually to draw the various straight lines, which are apt to become confusing if many tree measurements are available. Instead, a straight edge may be laid across the proper values and its intersections with the intermediate axis noted.

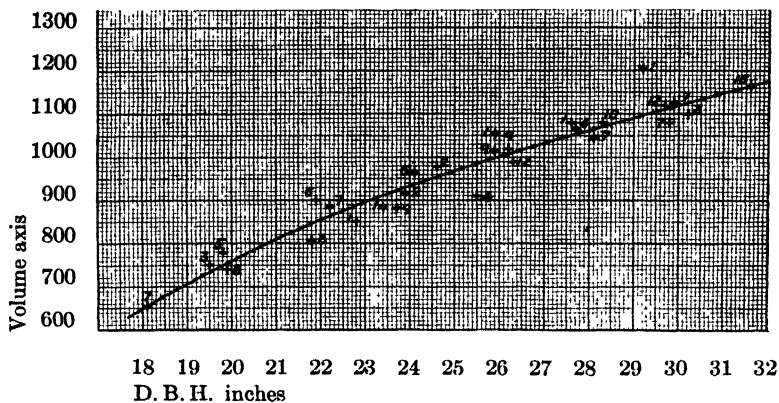


Figure 3

The graduating curve.

There are several advantages in this method of preparing a volume table. In the first place, the curve drawing is simplified. In place of the system of curves which have to be harmonized in the usual plan only a single graduating curve need be drawn, and since this is based on all of the tree measurements available it is much better defined and more easily and accurately located. As compared with the frustum form factor method, which also uses a single curve, the use of the alinement method saves considerable time, since the calculation of the form factors is avoided; the result is, however, practically identical, for if the frustum form factors of such a table as Table III be calculated they will be found to vary with diameter but not materially with height.

Secondly, extrapolations are easily (perhaps almost too easily) made, especially in height, and with far more certainty than is possible by the normal system of curve extension.

Lastly, the resulting alinement chart can be read with great accuracy for fractions of inches in diameter and fractions of logs in height.

This is not true of the conventional method, where graphic interpolations between the harmonized curves are both slow and inaccurate and where arithmetical interpolations in the final table are exceedingly laborious. For certain problems of forest mensuration this advantage is highly important, although it is of little weight in connection with ordinary timber cruising. The method appears superior in accuracy to the ordinary plan, especially where the amount of data available is limited. Volume tables have been made by both methods from tree data for three species, including the species used in illustrating this paper. The results appear as follows:

Basic data	Aggregate difference between all trees as actually scaled and as read by table		Average deviation between individual tree volumes as scaled and as read by table	
	Conventional	Alinement	Conventional	Alinement
145 trees, western larch.....	1.5%	0.2%	5.8%	5.1%
166 trees, western white pine.....	2.1%	0.3%	3.8%	3.9%
166 trees, white fir.....	0.5%	0.2%	7.1%	6.3%

It will be observed that the result by the alinement method is much superior as a whole for each species, and is better, on the average, in detail as well.

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**EQUILIBRIUM STUDIES WITH CERTAIN ACIDS  
AND MINERALS AND THEIR PROBABLE  
RELATION TO THE DECOMPOSITION  
OF MINERALS BY BACTERIA**

**BY**  
**DOUGLAS WRIGHT, JR.**

**UNIVERSITY OF CALIFORNIA PRESS**  
**BERKELEY, CALIFORNIA**

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INTRODUCTION

The importance of the bacterial population to the soil is well recognized. The rôle of micro-organisms in the processes of ammonification, of nitrification, and of nitrogen fixation has been the subject of so much investigation, and has been reviewed so often in the literature that it is generally accepted as fact. The influence of bacterial life, or of the end-products resulting therefrom, causing as it does the solution of necessary plant nutrients from the mineral particles within the soil, has been the object of much speculation, some of which has been substantiated by experiment. Aside from this effect of bacteria upon the mineral particles within the soil, there is some reason for believing that rocks may undergo disintegration and degradation into soil through the action of bacteria. This subject has been discussed and investigated at some length by various writers, whose work will be mentioned later.

The formation of the mineral portion of the soil is due to the operation upon the rock mass of three general factors, namely, changes in

physical environment, chemical action, and biological activity. The first of these exerts so apparent an influence upon rocks that it has long been recognized and subjected to careful investigation by geologists. As applied to soils, these effects belong in the realm of the soil physicist and therefore will not be considered in this paper.

The chemical agencies chiefly instrumental in breaking down and dissolving mineral material are water, solutions of varying amounts of  $\text{NH}_3$  and  $\text{CO}_2$ , various salts, organic acids, and organic compounds. The effect of solutions, especially of neutral salts, has been the subject of extensive investigation. This work will be reviewed later in the section of this paper dealing with method of attack and theory.

Certain biological activities are generally acknowledged to be operative in breaking down the rock mass and preparing it for use as a suitable medium for the growth of plants. Some of these are mechanical, such as the manifest action of roots in prying apart portions of rock. Other effects on rocks are far less easily discernible, due to the slight action of a vast population of microscopic flora found upon rock in all stages of its decomposition. The growth of algae, both alone and in their symbiotic relationships with the lichens, may aid, through the effect of respiration products, in the solution of minerals. It is likely, however, that the more important office of these simple green plants is to serve directly or indirectly as a source of energy for the growth of the still smaller organisms—the bacteria—in situations where the supply of organic materials is limited.

A statement concerning the effect of bacteria in rock decomposition was made by Müntz<sup>1</sup> as early as the year 1890. He found bacteria "in the denuded rocks of the Alps, the Pyrenees, the Auvergne, and the Vosges comprising the most varied mineralogical types: granites, porphyries, gneiss, mica schist, volcanic rocks, limestones, and sandstones, . . . Often the action is not confined to the surface, but extends into the depth of the rock mass. This is the case with the so-called rotten rocks of which the particles become disengaged and separate as is often seen in limestones, schists, and granites. . . . In decomposed rocks I have always verified the presence of nitrifying organisms."

Branner<sup>2</sup> takes issue with Müntz's assumptions, citing the need of bacteria for a large supply of oxygen and nitrogen, and their saprophytic habit as prohibitive of their growth to any extent upon or in rocks. Had Branner's statements been made some years later, they undoubtedly would have been modified by recent information concerning the nitrogen compounds of the fundamental rocks. The investigations of Hall and Miller<sup>3</sup> show that part of the nitrogen in certain

clay\* soils may have been derived from the nitrogen compounds in the rocks from which those soils were formed.

Merrill<sup>4</sup> mentions bacteria as possible agencies in the decomposition of rocks.

Renault<sup>5</sup> found bacteria present in coal, and postulates their action in coal beds as that of the transformation of carbonaceous material into methane and hydrogen.

Holland<sup>6</sup> suggests that the phenomenon of laterization may be due to the action of bacteria, possibly to some specific organism allied to the sulfur and iron bacteria, and gives certain observations which lead to this belief.

Lacroix<sup>7</sup> makes the following statement: "The bare rocky islet of Cabras, near San Thome in the Gulf of Guinea, is covered with a mantle of slightly ferruginous aluminum phosphate which is sometimes several centimeters thick. This has originated from the interaction of some of the products of bird guano and the underlying rock, aided, doubtless, by microbes."

All the conclusions in the literature mentioned thus far are of a conjectural character and are the result of observation only, no controlled or investigational work having been presented in support of the opinions offered. The most important systematic investigation of the action of bacteria on rocks was undertaken by K. Bassalik<sup>8</sup>, and is reported by him in two papers, *The Decomposition of Silicates by Soil Bacteria*, and *The Decomposition of Silicates by Soil Bacteria and Yeasts*.

The first of these papers is more or less preliminary, the author drawing the conclusion that bacteria are able to derive their necessary mineral nutrients from the feldspars, and that appreciable quantities of unweathered orthoclase are dissolved by bacteria, probably by means of CO<sub>2</sub> produced by the latter.

The second paper reports an elaborate study of the effect of the growth of several organisms upon various minerals. *B. extorquens*, several of the nitrifying organisms, butyric acid bacteria, and yeasts were tried upon twelve widely varying silicates and upon apatite. A partial summary of Bassalik's results is given here:

1. Bacteria are able by means of their products of respiration to cause a significant solubility of pulverized silicates.
2. Those which produce organic acids, as *Clostridium Pasteurianum*, influence more strongly the solubility of the silicates.
3. In the action of micro-organisms upon rocks, the intensity of contact of the organism and the mineral to be dissolved is of greater importance than the various agents of solubility.
4. Thus, *B. extorquens*, which produces only CO<sub>2</sub>, has the strongest solvent effect through its close and firm envelope of the mineral particles.

5. Yeasts, which produce much more  $\text{CO}_2$  in cultures than *B. extorquens*, cause a smaller solubility because of the absence of the close contact with the mineral particles.

6. The nitrite bacteria are also able to effect a significant solubility of silicates as the result of their physiological property of oxidizing  $\text{NH}_3$ , but they affect those minerals rich in *alkaline earths* much more than those rich in *silicates*.

7. The significant solubility of apatite seems to be a property only of those bacteria which produce organic acids, for this mineral is dissolved only in moderate degree by those organisms which produce  $\text{CO}_2$ .

8. In the filtrates of the bacterial cultures, especially with *B. extorquens*, can be recovered all the chemical constituents present in the minerals experimented with. Those most easily going into solution are the alkalis, then the alkaline earths and iron, silicic acid much less, and clay the least.

This summary presents some interesting conclusions, and a close review of the paper shows that they are the result of careful work. Bassalik, however, does not get at the fundamental causes of the differences in the effects of organisms. This investigator refers, in conclusions 3, 4, and 5, to the closeness of contact of organisms to the mineral as an important factor in determining the magnitude of the action of *B. extorquens*; but he does not offer plausible proof of this assumption, and it would seem that his conclusion concerning this point may be erroneous. If we have in solution  $\text{H}_2\text{CO}_3$  from the production of  $\text{CO}_2$  by *B. extorquens*, the concentration of the acid should depend upon the partial pressure of the  $\text{CO}_2$  above the liquid and the rate of  $\text{CO}_2$  production by the organism. The same should be true with the yeast, and as the yeast, according to Bassalik's own statement, produces  $\text{CO}_2$  more rapidly than does *B. extorquens*, and if, as he also states, the solubility is effected by the concentration of  $\text{CO}_2$ , the yeast should effect the greater solution of the mineral. This should be true, both in the solution culture and in the solution film surrounding the mineral particles, where the organisms are grown upon the moist mineral. Thus it would seem that any greater effect of *B. extorquens* should be attributed to some specific action on the mineral, such as oxidation, hydration, etc., rather than to  $\text{CO}_2$ . Furthermore, the bacterial envelope, which is referred to as enclosing the mineral particles, may consist of a gelatinous coating produced from the mineral particle itself, rather than of an aggregate of bacteria (this coating being greater where the action upon the mineral is greater).

In 1915 T. Kawamura<sup>9</sup> described an organism found in some volcanic material upon one of the mountains of Japan, at an altitude of 6,600 feet. This organism is of special interest as one which has a specific action upon a silicate material. It forms a zoogloeic mass, the ash of which contains an unusually large amount of silica, 8.873 per cent. Kawamura proposed the name *Volcanothrix silicophila* for the organism.



A comprehensive discussion of the action of bacteria upon minerals would not be complete without some reference to the action of certain organisms upon the iron, sulfur, and phosphorus compounds found in rocks and soils. However, since it is the purpose of this paper to deal with an entirely different phase of the subject, a brief reference to the bacterial processes affecting these compounds will suffice.

Lipman and McLean<sup>10</sup> studied the effect of the oxidation of sulfur upon rock phosphate and found appreciable amounts of the phosphate dissolved through the action of the resulting acid.

Stoklasa<sup>11</sup> found marked solubility of bone meal through the action of soil bacteria and attributed it to the action of enzymes upon the bone meal.

Koch and Kroeber<sup>12</sup> and later Kroeber<sup>13</sup> determined the solubility of different forms of phosphate in the acids produced by the growth of soil and sewage organisms upon dextrose. Kroeber concluded that the acids produced by bacteria and yeasts in the soil may be of great importance in rendering phosphate soluble. In cultures where  $\text{CaCO}_3$  was present little or no phosphate was made soluble.

Sackett, Patten, and Brown<sup>14</sup> in a somewhat similar work found that there was a decided solution of the insoluble phosphate when bacterial growth was accompanied by acid formation. They believed that acid is not the sole solvent.

Hopkins and Whiting<sup>15</sup> discuss the effect upon rock phosphate of the nitrous acid produced through the oxidation of  $\text{NH}_3$  by *Nitrosomonas*.

## OBJECT OF INVESTIGATION

Bacteria may effect the solution and disintegration of minerals in at least two ways:

1. Through the oxidation or reduction of one or more of the constituents of the mineral by specific organisms.
2. By the action of some end-product of bacterial activity: i. e., H ion resulting from acid produced, or OH ion from the production of  $\text{NH}_3$ .

In the present investigation some of the fundamental considerations in connection with the second phase of the subject were studied, the work being limited to the effect of acid end-products. In none of the work reviewed in the foregoing section have attempts been made to obtain results which may be used to determine whether the action of bacteria upon minerals may follow the usual chemical laws, or at least

present some constant relationship which may be expressed in an empirical formula. The work in hand has had for its object the procurement and interpretation of data suitable for the confirmation of some such relationship.

## METHOD OF ATTACK AND THEORY

In order to obtain such data it was found necessary to use a different method of attack from that usually pursued in a bacteriological problem. The most common approach to such a problem is by the determination, either in solution culture, in sand culture, or in culture upon the moist pulverized mineral itself, of the amount of material made soluble by the growth of certain organisms. This method gives a series of isolated results, which, though no doubt interesting in themselves, are entirely unrelated either among themselves or to any factor which may control the magnitude of the bacterial effect. In dealing with the phase of the problem studied in this paper, a different method is employed, a method by which it is hoped to show a certain relationship between H ion produced by bacteria and the amounts of bases brought into solution.

The magnitude of the effect of bacterial end-products upon a mineral will depend upon the equilibrium involving that end-product and mineral. As stated before, in this study it is elected to deal with cases in which acids are the end-products in question. Therefore, it was deemed necessary first to study the equilibria of certain acids, used over a wide range of concentrations, with certain minerals. The object of these equilibrium studies was to compare the H ion concentrations of the acids, at the various molar concentrations, with the amounts of material which are brought into solution, so to speak, by these H ion concentrations. Later, studies were made of the H ion production by certain organisms, and of the equilibria involving these acids and the minerals, the H ion and the amounts of material in solution being determined.

There is an extensive literature dealing with the equilibria of various soils and minerals in contact with solutions of acids, of bases, and of salts. This literature deals largely with the absorption of bases by soils and minerals, and with the exchange of bases between solution and soil or solution and mineral. In nearly every instance, however, the data are insufficient to warrant their use for substitution in formulae.

The earlier work is so ably and completely reviewed by Sullivan,<sup>16</sup> in his consideration of *The Interaction between Minerals and Water Solutions*, that it seems advisable to refer the reader to that excellent résumé rather than to attempt a repetition here. This review covers the work of Thompson,<sup>17</sup> Way,<sup>18</sup> Eichhorn,<sup>19</sup> Henneberg and Stohmann,<sup>20</sup> Lemberg,<sup>21</sup> Peters,<sup>22</sup> Liebig,<sup>23</sup> Rautenberg,<sup>24</sup> Van Bemmelen,<sup>25</sup> Armsby,<sup>26</sup> and Boedeker,<sup>27</sup> and deals largely with the controversy of the physical process of adsorption versus chemical reaction as the cause of the absorption and exchange of bases in soils.

The work of Ditttrick<sup>28</sup> is not included above. His work is reported in two papers, and covers experiments with a granite and an amphibole paridotite, and solutions of KCl, NaCl, NH<sub>4</sub>Cl, CaCl<sub>2</sub>, MgCl<sub>2</sub>, KNO<sub>3</sub>, K<sub>2</sub>SO<sub>4</sub>, and K<sub>2</sub>CO<sub>3</sub>, in the concentrations N/1, N/10, and N/100. He found Ca and Mg dissolved by the solutions, the least by N/1 solution, more by the N/10 solution, and most by the N/100 solution. The amount of exchange was greater with the more decomposed rock. Repeated extraction with solutions removed roughly twice as much material as a single extraction.

The action of an acid upon a silicate is really an exchange of H ion for any of the bases which come into solution through its action. This exchange is a reversible chemical reaction, and as such should conform with the chemical laws applicable to such reactions.

Let us consider a simple case of reversible reaction, or balanced action, that of the union of hydrogen and iodine to form hydriodic acid:  $H_2 + I_2 \rightleftharpoons 2HI$ .

In this reaction there is a point of equilibrium which is represented by the equation:

$$\frac{C_{H_2} \times C_{I_2}}{C_{HI}^2} = \frac{k_1}{k} = K.$$

This is an example of homogeneous equilibrium, involving the gaseous phase only.

A somewhat different case is encountered with the decomposition of calcium carbonate into carbon dioxide and calcium oxide:



Here we have both gaseous and solid phases. The amount of gas taking part in the reaction may be measured by its pressure, but the solid must be considered in a different light. This reaction may be considered as taking place in the gaseous phase, the solids present furnishing a constant supply of CaCO<sub>3</sub> and CaO vapor. Then if C is the

pressure of  $\text{CaCO}_3$ ,  $C_1$  the pressure of  $\text{CaO}$ , and  $c$  the pressure of  $\text{CO}_2$  at equilibrium, the equation at equilibrium is:

$$kC = k_1 C_1 c, \text{ or } c = \frac{kC}{k_1 C_1} = K$$

Going farther we have the following as a reaction in which we have a solid and a gas on both sides of the equation:



Let  $c$  be the concentration of  $\text{H}_2\text{O}$ ,  $C$  that of  $\text{Fe}$ ,  $C_1$  of  $\text{FeO}$ , and  $c_1$  of  $\text{H}_2$ . At equilibrium we then have the equation:

$$\begin{aligned} k c C &= k_1 c_1 C_1 \\ \text{and } \frac{c}{c_1} &= \frac{k_1 C_1}{k C} = K \\ \text{or } \frac{c}{c_1} &= K \end{aligned}$$

Now applying this last equation to a case where we have a solution of a salt acting upon a solid to form another salt in solution and a solid we will take the following reaction:



Let  $C$ ,  $c$ ,  $c_1$  and  $C_1$  be the respective concentrations of the reacting substances. Then from the above equation

$$\begin{aligned} \frac{c}{c_1} &= K, \\ \text{or } \frac{C_{\text{Na}_2\text{CO}_3}}{C_{\text{Na}_2\text{SO}_4}} &= K. \end{aligned}$$

This last equation shows that the equilibrium point is measured by the ratio of concentrations of the soluble reacting materials. As stated by Walker:<sup>29</sup> "The active masses of the barium salts may be accounted constant in the reaction, for although they are generally spoken of as 'insoluble', they are in reality measurably soluble in water. The aqueous liquid in contact with them will be and remain saturated with respect to them, i.e., their concentration and active mass in the solution will be constant. The equilibrium will thus be determined by a certain ratio of the concentrations of the soluble sodium salts, independent of what the actual values of the concentrations may be."

A mineral in contact with an acid solution is very similar to the last case cited above, and at any concentration of the acid the equilibrium should be measured by the ratio

$$\frac{\text{concentration of the acid}}{\text{concentration of the material in solution}} = K$$

when these are measured at equilibrium.

The H ion is usually assumed to be the measure of the active acid. In acid solution the H ion concentration is some function of the molar concentration of the acid. Since gas chain measurements yield values which approach the theoretical H ion concentration, determinations of the H ion concentrations,  $C_h$ , by this method may be substituted for "concentration of acid" in the above formula and the ratio

$$\frac{C_h}{C_{\text{material in solution}}}$$

will be constant.

When dealing with materials as complex in their chemical structure as are minerals, it is recognized that a rigid adherence to the theoretical laws can not be expected, and it becomes necessary, therefore, for a comprehensive knowledge of such reactions as are considered in this paper, to resort to certain empirical formulae. It is obviously impracticable to attempt to consider in the term "concentration of material in solution," as used in the formula above, all the bases which may be present in the solution in contact with the mineral at equilibrium. These considerations lead to the assumption that any one of the bases may be taken as measuring the magnitude of the action of the acid on the mineral. Consequently, it is suggested that the formula thus far developed theoretically, be changed to the empirical formula

$$\frac{C_h}{C_a} = K,$$

where  $C_h$  is the concentration of hydrogen ion, and  $C_a$  represents the molar concentration of Ca, Mg, Fe, or K in solution at equilibrium with the acid.

Since it is desired to study the initial H ion concentration with respect to Ca, Mg, Fe, or K in solution, it becomes necessary to add a still further modification to the empirical formula—namely, the expression of the initial H ion concentration in terms of, or as some function of, the H ion concentration at equilibrium. It is found that this is an exponential function of the H ion concentration at equilibrium. (See fig. 0.) In this figure,  $\log. C_h$  of the acid alone is plotted against  $\log. C_h$  of acid in contact with the mineral. The resulting graph is a straight line, indicating that the ratio is constant, at least over a certain range of concentrations. Thus  $C_h$  of the acid alone is a logarithmic or exponential function of  $C_h$  of the acid in contact with the mineral, or  $C_h = C_h^x$ . Then the equation  $\frac{C_h}{C_a} = K$  becomes  $\frac{C_h^x}{C_a} = K$ , where  $C_h^x$  is the

initial H ion concentration of the acid,  $C_a$  the concentration of Ca, Mg, Fe, or K in solution at equilibrium, and K is a constant. This equation is the one used in the consideration of the data contained in this paper.

The formation of acid as the end-product of bacterial activity is a property common to many organisms including many which are commonly found in soils. Acid production by bacteria has been the subject of many investigations. It is used as a means for identifying the various members of the Colon group of bacteria, and consequently the fermentation of sugars by this group has been widely studied. A review of the entire field will not be attempted, but it is well to mention work having a more or less direct bearing upon the subject in hand.

Harden<sup>30</sup> studied the *Chemical Action of B. coli communis and Similar Organisms on Carbohydrates and Allied Compounds*. He found that the lactic acid produced never exceeds one-half of the sugar fermented. The amount of acid formed varies with the different sugars.

In a later study with Penfold<sup>31</sup> he used *B. coli* on a medium composed of 2 per cent glucose and 1 per cent peptone. He gets of alcohol, acetic acid, formic acid, CO<sub>2</sub>, lactic acid, and succinic acid, respectively 17.22 per cent, 20.60 per cent, 2.55 per cent, 17.30 per cent, 40.60 per cent, and 4.80 per cent of the sugar used. With a selected strain of *B. coli*, the lactic acid reaches 70 per cent of the amount of sugar used.

Michaelis and Marcora<sup>32</sup> find that the highest degree of acidity produced by *B. coli* at 37° C. is  $1 \times 10^{-5}$ .

In the lactic acid fermentation of sugars, Claffin<sup>33</sup> notes the formation also of formic, propionic, and acetic acids, the acetic acid formation depending upon the degree of aeration. Ninety-five to 97 per cent of the sugar may be converted into lactic acid, with a very low production of volatile acids, not over one-half per cent. He claims that the nature of the acid produced depends upon the organism and not upon the nature of the medium.

In the present work it must be shown what is the amount of acid, or rather the H ion concentration, produced by the organisms upon the carbohydrate media used, and what is the effect of this concentration upon the minerals. Is this effect similar to that of the acids alone?

## EXPERIMENTAL METHODS

As suggested in the foregoing section, the experimental work is divided into two parts, the first consisting of the equilibrium studies with the minerals and acid solutions, the second of bacteriological studies.

*Equilibrium studies.*—The acids used were hydrochloric, sulfuric, oxalic, phosphoric, lactic, formic, and acetic. It will be observed that these acids vary in the degree of dissociation for any given concentration, hydrochloric acid being the most highly dissociated, and acetic acid being the least ionized. The minerals were calcium silicate, orthoclase feldspar, biotite, and granite. They were ground in a ball mill to pass a 200-mesh sieve. The acids were used in the concentrations: N/5, N/25, N/50, N/100, N/250, N/500, N/1,000, N/2,000, N/5,000, and N/10,000.

The work was carried on at room temperature. The equilibrium studies were arranged in four series, one for each mineral, and each series contained a sub-series for each acid. 200 cubic centimeters of solution were thoroughly shaken with 5 grams of mineral, Jena glass-ware being used. The solutions were allowed to remain in contact with the mineral for three days, which time is shown in the following table to be sufficient for equilibrium.

TABLE SHOWING THE EFFECT OF THE TIME OF CONTACT  
UPON THE H ION CONCENTRATION.

Orthoclase + N 5 HCl Days	H ion concentration
1	.709 x 10 <sup>-1</sup>
2	.636 x 10 <sup>-1</sup>
3	.615 x 10 <sup>-1</sup>
4	.656 x 10 <sup>-1</sup>
5	.615 x 10 <sup>-1</sup>

As much as possible of the supernatant solution was then pipetted off and filtered through a Whatman No. 42 filter paper, an unfiltered portion being taken, however, for the H ion determination. In an aliquot of the filtered solution, calcium, iron, magnesium, and potassium were determined, the calcium and iron by titration with potassium permanganate, the magnesium and potassium gravimetrically as the pyrophosphate and chloroplatinate respectively.

The H ion determinations were made by the use of the hydrogen electrode, with the same modifications as were used by Sharp and Hoagland<sup>34</sup> for soils. These determinations are made both on the acid solutions, and on the acids in contact with the minerals.

A question arose as to the possibility of the formation of a gelatinous coating upon the surface of the mineral, which would hinder the further action of the acid, and prevent attainment of equilibrium. In order to ascertain whether the amount of shaking had been sufficient to remove this film and allow the reaction to come to equilibrium, the following experiment was proposed. A 5-gram portion of mineral was shaken with 200 cc. of N/5 HCl, as in the experimental procedure. The mineral and solution were then poured upon a filter paper, and the mineral was dried and then ground in a mortar. H ion determinations were made upon the filtrate. The filtrate was poured upon the dried mineral and allowed to remain for three days with frequent shaking. H ion determinations were again made. The following data show that there is no significant change in H ion concentration in the second contact of the solution with the mineral:

	H ion Conc.
Calcium silicate +N 5 HCl... 1st contact ; ...	$0.607 \times 10^{-1}$
Calcium silicate +N 5 HCl....2d contact... .	$0.607 \times 10^{-1}$
Labradorite +N 5 HCl... 1st contact . ...	$0.797 \times 10^{-1}$
Labradorite +N 5 HCl.. 2d contact .....	$0.828 \times 10^{-1}$

*Bacteriological work.*—Three organisms were used, *Azotobacter*, *Bacillus coli*, and *B. lactis acidii*. The first of these was chosen because of the very high H ion concentration which it produced upon dextrose solution, nearly  $1. \times 10^{-1}$  as determined by Dr. Waynick in this laboratory. *B. coli* is referred to in the literature previously cited as producing large amounts of acid, and *B. lactis acidii* was taken as a typical acid producer. *Azotobacter* were grown upon 2 per cent dextrose solution, *B. coli* in a solution of 2 per cent dextrose and 1 per cent peptone as used by Penfold, and *B. lactis acidii* in 1 per cent dextrose. The work was arranged in three series, one for each organism. Each series contained five cultures, each culture containing 1,000 cc. of solution in 1,200 cc. Florence flasks. One culture contained no mineral. The other cultures contained 25 grams of mineral each, one with calcium silicate, one with orthoclase feldspar, another with biotite, and one with granite. These cultures were run for a total time of sixteen days, H ion determinations being made at 1, 2, 3, 5, 7, 9, 11, and 16 days, and on each of these days 100 cc. of solution was removed for the determination of calcium, iron, magnesium, and potassium. The cultures were grown at a temperature of 28° C. and the customary bacteriological precautions were observed throughout the work.



## DATA

The data obtained by the methods given above are reported in tables 1 to 38 inclusive. The amounts of calcium, magnesium, iron, and potassium are calculated and expressed as moles per liter. Both the initial and final H ion concentrations are also reported as gram molecules per liter. For the convenience of the reader, the logarithms of these numerical values are given in adjacent columns.

Accompanying each table is a graphical representation of the relation between certain series of values given in that table. (Owing to a loss of material during the analysis, tables 3, 9, and 22 are incomplete; there are, therefore, no graphs for these tables.) The logarithms of the H ion concentrations,  $\log. C_h$ , are plotted along the ordinates, and the logarithms of the concentrations of Ca, Mg, Fe, or k,  $\log. C_a$ , along the abscissas, and the average curve is drawn through the points thus obtained.

In the section of this paper dealing with "Method of Attack and Theory," certain assumptions are made and ultimately expressed in the empirical formula,  $\frac{C_h^x}{C_a} = K$ . By substitution of the experimental data in this formula, the values of the constants  $x$  and  $K$  may be calculated. If these values of  $x$  and  $K$  are constant for a given series, then the ratio,  $\frac{C_h^x}{C_a}$ , is constant for that series, and the plotted graph representing that ratio will be a straight line, or conversely, a straight line curve indicates that  $x$  and  $K$  are constant. The straight line graph expresses a direct ratio between series of values, these values being, in this case, the logarithms of  $C_h$  and of  $C_a$ .

The exponential constant  $x$  and the reasons for its use have been discussed previously. It expresses the relation of H ion at equilibrium to the initial H ion concentration of the acid. That this relationship is of an exponential character may be due to chemical reaction, to adsorption, or to a combination of these phenomena. It is not proposed, however, to differentiate here between adsorption and chemical reaction, the purpose of this work being to provide a means, empirical if necessary, of accounting for the magnitude of the action of acids upon minerals.

The constant  $K$  is the numerical expression of the ratio of the logarithm of the initial H ion concentration to the logarithm of Ca, Mg,

Fe, of K brought into solution, so to speak, by that H ion concentration. The numerical magnitude of K for any table represents the slope or inclination of the graph plotted from that table. The range of values for K may be very large, varying from infinity, for a horizontal line, to zero, for a vertical line.

Since it is possible to draw a straight line averaging the points plotted from the experimental data, the graphical method is employed for obtaining values of  $\log. C_h$  and  $\log. C_a$  for substitution in the equation for the calculation of x and K. By this means average values of x and K may be computed without resorting to a calculation of all the possible combinations of equations for which data are available. The use of this procedure eliminates a large part of the tedious mathematical routine, and it is recognized as yielding averages sufficiently close to the statistical average to serve the purpose contemplated in this paper. If the above preliminary remarks are borne carefully in mind, the following consideration of the groups of tables and graphs will be clear.

Tables 1 to 7 contain the data for the equilibria between calcium silicate and the acids. The figures accompanying these tables are sufficient to show that the graph for any given series is a straight line. As stated before, this signifies that x and K are constant for each series, and that the reaction of the acid with the mineral takes place in accordance with the formula  $\frac{C_h^x}{C_a} = K$ . The slope of the graphs, however, seems

to become less for the equilibria involving the less dissociated acids, the slope for acetic acid being much less than that for hydrochloric acid. As explained before, this difference in slope is indicated by the following numerical values for the constant K calculated from the graphs, the slope becoming less as K increases:

HCl .....	K = 0.01391
Sulfuric acid .....	K = 0.2642
Phosphoric acid .....	K = 0.4448
Lactic acid .....	K = 14.86
Formic acid .....	K = 335.5
Acetic acid.....	K = 941.2

Since, as stated before, the graphs represent ratios of  $\log. C_h$  to  $\log. C_a$ , and since this ratio varies with the slope of the graph, it would seem, from a comparison of the curves for HCl and acetic acid, that an acid such as HCl, which is highly ionized, brings smaller amounts of material into solution per unit increase of H ion than do those acids, acetic for instance, which have a lower ionization constant. This apparent difference in the action of various acids is due undoubtedly to

the fact that the commercial calcium silicate was used for this work, and that it contained considerable quantities of  $\text{CaCO}_3$ , as shown by the marked effervescence which occurred when the acid was added to this material. The loss of  $\text{CO}_2$  from the system undoubtedly affected the true equilibrium, and this apparently greater action of the less dissociated acids is the result. As will be seen in the considerations which follow, this difference in effect between various acids occurs only with calcium silicate.

In tables 1 to 7, as well as in those which follow, there are no data for calcium where oxalic acid is used, because of the insolubility of the resulting calcium oxalate. This fact is mentioned again, and its importance is emphasized further, in connection with a general statement concerning the action of the H ion concentration of acids upon minerals.

The data for the various acids and orthoclase are found in tables 8 to 12 inclusive. From a comparison of the corresponding graphs, it is seen that they are quite steep, and that all have approximately the same slope. This observation is verified by a consideration of the constant,  $K$ , as calculated for the various members of this group of tables. The fact that  $K$  is very small is evidence that the graphs approach the perpendicular, and when it is remembered that the values for  $K$  range from zero to infinity for a change of 90 degrees in slope, it is obvious that the very small range of values for  $K$  given here, 0.00002661 to 0.000001427, represents a very small difference in the slopes of the various curves. The fact that  $K$  is constant for each series, and that the corresponding graph is a straight line, goes to show that the calcium coming into solution is a logarithmic function of the initial H ion concentration of the acid, and that the assumptions expressed in the formula  $\frac{C_h^x}{C_a} = K$  are verified by experimental evidence

Further proof of these assumptions is offered in the next group of tables, numbers 13 to 19 inclusive, which give the data for the acids in equilibrium with biotite. Data for calcium, magnesium, and potassium in solution are reported. As in the previous series of tables, the graphs for calcium are straight lines and have about the same slope, the extreme range of values for  $K$  being from 0.00005790 to 0.000001071. The constant  $K$  for the magnesium determinations is more variable, but still no large discrepancy is apparent, the values ranging from 0.04373 to 0.000008395.

A deviation from the straight line graph is encountered in the figures and tables expressing the equilibria for granite and the acids,

tables 20 to 26 inclusive. When the logarithms of the data for iron are plotted against the logarithms for H ion concentrations and the lines are drawn through the points thus obtained, the resulting curves are not straight lines. (See figures 20 to 26.) The flatter portion of the curve occurs in every instance at approximately the value,  $\bar{3}.0$ , represented by that logarithm of the H ion concentration, and probably is due to the formation of another compound of iron at that concentration. Since these curves are not straight lines, it is obvious that no constant values for  $x$  and  $K$  may be calculated therefrom, and furthermore it may be inferred that any iron compounds in the mineral do not react with the acids in accordance with the proposed formula. The curves for calcium, however, are straight lines, thus affording still further proof that the assumptions regarding the nature of such reactions are correct as far as calcium is concerned. The graphs have nearly the same slope throughout the series of figures, the values for  $K$  ranging from 0.000001328 to 0.000007799.

In a general survey of the graphs thus far discussed, instances may be observed in which certain plotted points are far from coincident with the straight line graph. In certain cases, these discrepancies represent error in the determinations. Where they appear in the lower portion of the graph, however, the last two or three points dropping below the curve, they occur because the lower limit of the determination has been reached, and no smaller amounts can be determined with any degree of accuracy.

A consideration of the meaning and the possible relationships of the results thus far reviewed is not inappropriate at this point. It is the opinion of the writer that that type of investigation is the most valuable which has for its object the procurement of data which are related, either among themselves, or to certain controllable factors, and which may be taken as the basis for, or in verification of, some general law suitable either for the explanation of certain phenomena or for direct application in the prediction of future results. Thus a general consideration of the tables and figures leads to the following remarks.

All the straight line graphs for a given mineral, excepting calcium silicate, the deviation of which has been explained, have practically the same slope and give nearly the same values for  $x$  and  $K$ . It follows therefore, that if the curves for one mineral in contact with the various acids be superimposed one upon the other, they will all fall practically in the same straight line. This would seem to indicate, for a given mineral, and within the limits of the concentrations used, that the amount of calcium, magnesium, or potassium coming into solution is a function

of the H ion concentration of the acid, regardless of the nature of the acid used, except, and this exception is extremely important, in those cases where the acid forms compounds which are less soluble at any given H ion concentration than the compounds in the mineral itself. The importance of this exception must be emphasized, and it is illustrated in a very striking manner by the different series with oxalic acid where only traces of calcium are found in solution. It is recognized that this illustration represents an extreme case and that other so-called insoluble compounds may approach the mineral compounds in solubility.

The objection was raised to the foregoing generalization that a N/100 solution of acetic acid, for instance, contains the same total molar concentration of hydrogen as a N/100 hydrochloric acid solution, regardless of the relative H ion concentration of these acids. The hydrochloric acid is, of course, the more highly dissociated acid, but will not the slightly dissociated acetic acid continue to give off H ion as that already in solution combines with the mineral, and should not the ultimate result be the same with the acetic as with the hydrochloric acid for a given molar concentration? That this objection is not substantiated by fact is due doubtless to the following reason. In general the salts of acetic acid are much more highly dissociated than is the acid itself. Consequently, the acetates formed by the contact of acetic acid with the mineral will be fairly highly dissociated, thus supplying the solution at equilibrium with a certain concentration of acetic ion. The presence of this acetate ion will depress or prevent the further ionization of the acetic acid in solution, and thus practically limit the action of the acetic acid to its original H ion concentration. This same explanation will hold for other slightly dissociated acids.

The general relation existing between the H ion concentration of acids and the amounts of Ca, of Mg, or of K in solution, may have the following practical application. It is desired to determine the effect of certain acids upon a mineral. This mineral may be studied in equilibrium with different concentrations of HCl, and the logarithmic graph constructed as in the foregoing mineral series. Any point on this graph represents a ratio of log. H ion to log.  $C_a$ , or whatever base it is desired to consider, in solution. Thus, by determining the H ion concentration of the acid whose action it is desired to predict, we may, by referring to the graph, estimate the magnitude of the effect of the acid upon the mineral, at least within certain limits already discussed. The application suggested above may be made to the effect of acids produced by bacterial growth, and in the prediction of their action upon a given mineral.

The above application is suggested merely as a possibility, and it is fully recognized that there is room for much further study and research before the existence of such a general relationship can be definitely established. It must be emphasized also that the constants for one mineral and one set of conditions can not be applied directly to another mineral and a different set of conditions. The constants must be determined, and the resulting graph constructed for every application of the relation suggested.

The data obtained from the bacterial series still await consideration. As expressed in tables 26 to 38, and in the corresponding figures, the action of the acids produced by bacteria seems to differ in magnitude from the action of the acids used in the foregoing series. In regard to this difference, it should be observed that  $C_h$ , the H ion concentration, was determined, for the bacterial series, in solution cultures with no mineral present. Had the bacterial growth been stopped immediately following the H ion determination, and had this solution then been brought into contact with the mineral, the magnitude of the effect should have been comparable with that of the acid series. Instead, the amounts of material coming into solution were determined in a series parallel with the above, wherein bacteria were grown in solution in contact with the minerals. It had been assumed that the rate of H ion production would be the same, both in solution culture and in solution in contact with the mineral. This assumption was found to be incorrect. In the mineral cultures the acid was partly neutralized as produced, and the growth of the organism was not inhibited by the increasing concentration of acid as it was in the dextrose solution with no mineral. Consequently, the total H ion as produced in the mineral cultures, and indicated by the large relative amounts of material coming into solution in these cultures, was much greater than that produced in the parallel series without mineral. Since the data for H ion,  $C_h$ , as expressed in the tables, were obtained from the latter series, it is obvious that the graphs plotted from a ratio of H ion, as determined in dextrose solution, to material in solution, as determined in the mineral cultures, are not directly comparable with the graphs for the equilibria between acids and minerals.

The lack of agreement between the acid series and the bacterial series is made evident by a review of the curves and the corresponding constants for the bacterial series. The graphs are straight lines, but they have less slope than do those graphs for the corresponding mineral in equilibrium with the acids. For instance, the constant,  $K$ , for orthoclase and *Azotobacter* is 19.77 against a value approaching  $10^{-6}$  for

orthoclase and the acids, and the graph for the former has much less slope than the graph for the latter. The constant,  $K$ , for orthoclase and *B. coli* is 13,490, against  $10^{-6}$  for orthoclase and the acids. *B. lactis acidii*, which produced acid very slowly, is represented by graphs which more nearly resemble those of the acid-mineral series, and the value for  $K$  for calcium from orthoclase, for instance, is 0.0006652. Since discrepancies of the same general nature and magnitude as those just pointed out, are apparent in the other graphs and constants for the bacterial-mineral series, the reader is referred to the graphs and tables for further comparisons.

The important point brought out by the bacterial series is that the graphs for calcium, for magnesium, and for potassium are straight lines, and that  $x$  and  $K$  are constant for a given series. Thus it is shown that the reactions between minerals and the acids produced by bacterial growth, conform with the given empirical formula. Since this same formula has been successfully applied to the chemical equilibria between various acid solutions and the same minerals, it may be concluded that the action of bacterial end-products upon minerals, at least when these end-products are acids, is explainable upon the basis that it is a chemical reaction.

## SUMMARY

Equilibria of certain minerals and various concentrations of acids are studied.

Equilibria of the same minerals with solutions in which bacteria are producing acid are also studied.

The data obtained from the acid-mineral series are applied to the formula  $\frac{C_h^x}{C_a} = K$ .

It is found that the reactions occurring in the mineral-acid equilibria conform with the given formula.

It is suggested that a general relation exists between the initial  $H$  ion concentration of the acid and the amount of material which the acid brings into solution when in contact with a mineral.

A practical application of the relation just referred to, is suggested.

The data obtained from the bacterial studies are applied to the formula  $\frac{C_h^x}{C_a} = K$ .

The reactions occurring in the bacterial series also conform with the above formula.

It is concluded that the action of acid bacterial end-products upon minerals is explainable as a chemical reaction.

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## NOTE

The following legend refers to all the figures:

+ = Curve for Calcium.

○ = Curve for Magnesium.

⊕ = Curve for Potassium or Iron.

The numbers along the ordinates represent the logarithms of the H ion concentrations, or  $\log. C_h$ . Those along the abscissas measure the logarithms of the concentrations, or  $\log. C_a$ , of calcium, magnesium, iron, or potassium.

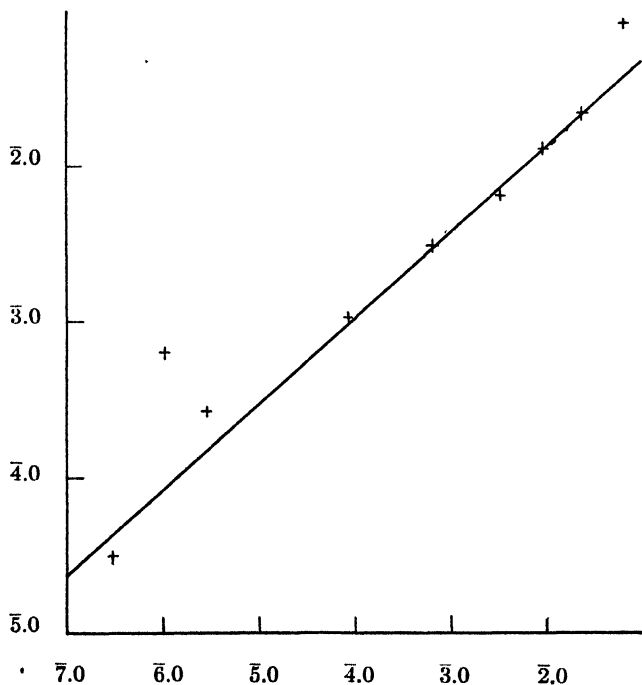


Fig. 0.

(See Table 8)

Relation of  $\log. H$  ion,  $HCl$ , to  $\log. H$  ion,  
 $HCl + Orthoclase$ .

TABLE I  
HYDROCHLORIC ACID AND CALCIUM SILICATE

Concentration HCl	C <sub>H</sub> , H ion Hydrochloric Acid	Log. H ion Hydrochloric Acid	H ion Hydrochloric Acid + Calcium Silicate	C <sub>a</sub> , Calcium, Mols. per Liter	Log. Calcium Mols. per Liter
N 5	0.0882	2.94547	0.0313	0.00306	3.485721
N 25	0.0218	2.33846	0.00000534	0.00218	3.338456
N 50	0.0135	2.13003	0.00000175	0.00121	3.02785
N 100	0.00685	3.83569	0.000000671	0.000685	4.835691
N 250	0.00321	3.50651	0.000000131	0.000376	4.575188
N 500	0.00114	3.05690	0.0000000842	0.000240	4.380211
N 1,000	0.000677	4.83059	0.0000000587	0.000159	4.201397
N 2,000	0.000281	4.44871	0.0000000965	0.000122	4.086360
N 5,000	0.0000326	5.51322	0.0000000719	0.000120	4.079181
N 10,000	0.0000192	5.28330	0.0000000620	0.000117	4.068168

Constants for the equation  $\frac{C_H^x}{C_a} = K$ :

$$x = 0.636$$

$$K = 0.01391$$

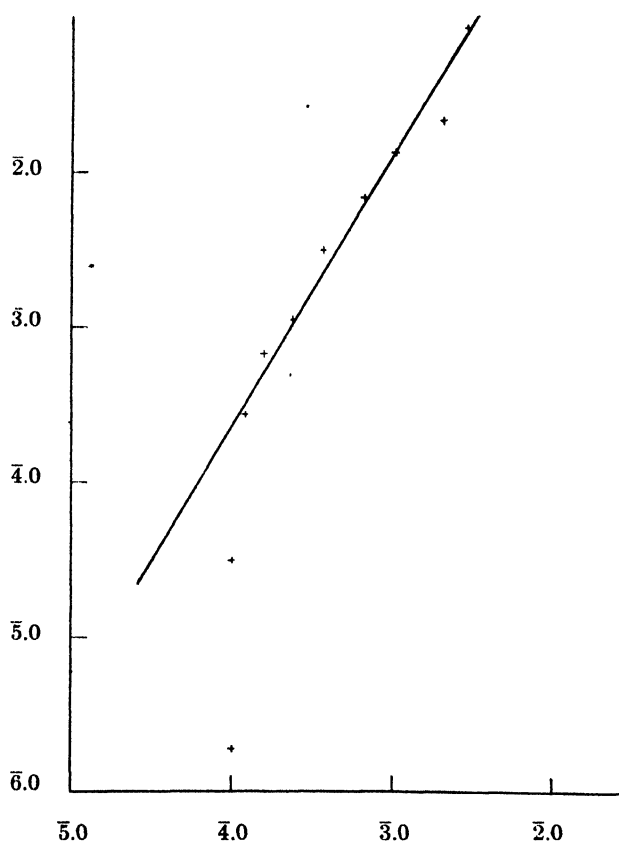


Fig. 1.  
Hydrochloric Acid and Calcium Silicate.  
(See Table 1)

TABLE 2  
SULFURIC ACID AND CALCIUM SILICATE

Concentration	$C_H$ , H ion Sulfuric Acid	Log. H ion Sulfuric Acid	H ion Sulfuric Acid + Calcium Silicate	$C_a$ , Calcium Mols. per Liter	Log. Calcium Mols. per Liter
N 5	0.0567	$\bar{2}.75358$	0.0300	0.00188	$\bar{3}.274158$
N 25	0.0186	$\bar{2}.28951$	0.00000579	0.00197	$\bar{3}.294466$
N 50	0.0130	$\bar{2}.11394$	0.000000450	0.00115	$\bar{3}.060698$
N 100	0.00632	$\bar{3}.80072$	0.000000203	0.000655	$\bar{4}.816241$
N 250	0.00296	$\bar{3}.47129$	0.0000000810	0.000356	$\bar{4}.551450$
N 500	0.00162	$\bar{3}.20952$	0.0000000719	0.000218	$\bar{4}.338456$
N 1,000	0.000931	$\bar{4}.96895$	0.000000180	0.000148	$\bar{4}.170262$
N 2,000	0.000600	$\bar{4}.77815$	0.0000000323	0.000099	$\bar{5}.995635$
N 5,000	0.000259	$\bar{4}.41330$	0.0000000235	0.000079	$\bar{5}.897627$
N 10,000	0.000174	$\bar{4}.24055$	0.0000000364	0.000089	$\bar{5}.949390$

Constants for the equation  $\frac{C_H^x}{C_a} = K$ :

By graphical average:

$$x = 0.799$$

$$K = 0.2642$$

By calculation:

$$x = 0.858 \pm 0.0074 \quad C. v. = 5.99\%$$

$$K = 0.7332 \pm 0.0313 \quad C. v. = 29.05\%$$

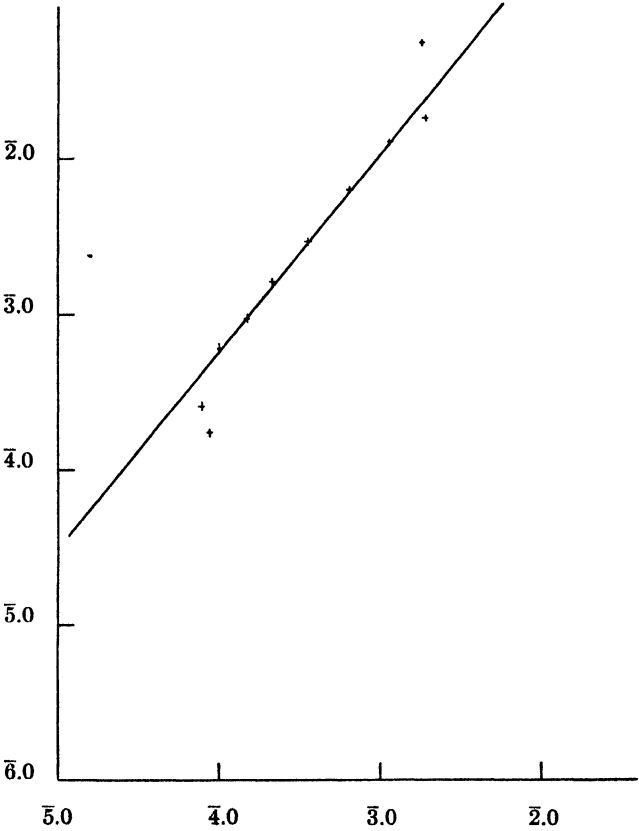


Figure 2.  
Sulfuric Acid and Calcium Silicate.  
(See Table 2)

TABLE 3  
OXALIC ACID AND CALCIUM SILICATE

Concentration	C <sub>H</sub> , H ion Oxalic Acid	Log. H ion Oxalic Acid	H ion Oxalic Acid + Calcium Silicate	C <sub>A</sub> , Calcium Mols. per Liter	Log. Calcium Mols. per Liter
N 5	0.0218	2.33846	0.0347	.....	.....
N 25	0.0110	2.04139	0.0000192	. ....	.....
N 50	0.00561	3.74896	0.00000175	. ....	.....
N 100	0.00347	3.54033	0.000000433	. ....	.....
N 250	0.00169	3.22789	0.0000000941	. ....	.....
N 500	0.00105	3.02119	0.0000000778	.....	.....
N 1,000	0.000554	4.74351	0.0000000719	. . .	.....
N 2,000	0.000317	4.50106	0.0000000637	. ....	.....
N 5,000	0.000132	4.12057	0.0000000544	. . .	.....
N 10,000	0.0000785	5.89487	0.000000469	. . .	.....

TABLE 4  
PHOSPHORIC ACID AND CALCIUM SILICATE

Concentration	C <sub>H</sub> , H ion Phosphoric Acid	Log. H ion Phosphoric Acid	H ion Phosphoric Acid + Calcium Silicate	C <sub>A</sub> , Calcium Mols. per Liter	Log. Calcium Mols. per Liter
N 5	0.0125	2.09691	0.0498	0.00307	3.487138
N 25	0.00632	3.80072	0.00000707	0.000872	4.940516
N 50	0.00424	3.62737	0.00000294	0.000525	4.720159
N 100	0.00273	3.43616	0.00000054	0.000366	4.563418
N 250	0.00144	3.15836	0.000000268	0.000208	4.318063
N 500	0.000762	4.88195	0.0000000941	0.000149	4.173186
N 1,000	0.000372	4.57054	0.0000000482	0.000109	4.037426
N 2,000	0.000213	4.32838	0.0000000350	0.000089	5.949390
N 5,000	0.0000642	5.80754	0.0000000235	0.000074	5.869232
N 10,000	0.0000431	5.63448	0.0000000395	0.000074	5.869232

Constants for the equation  $\frac{C_H^x}{C_A} = K$ :

$$x = 0.835$$

$$K = 0.4448$$



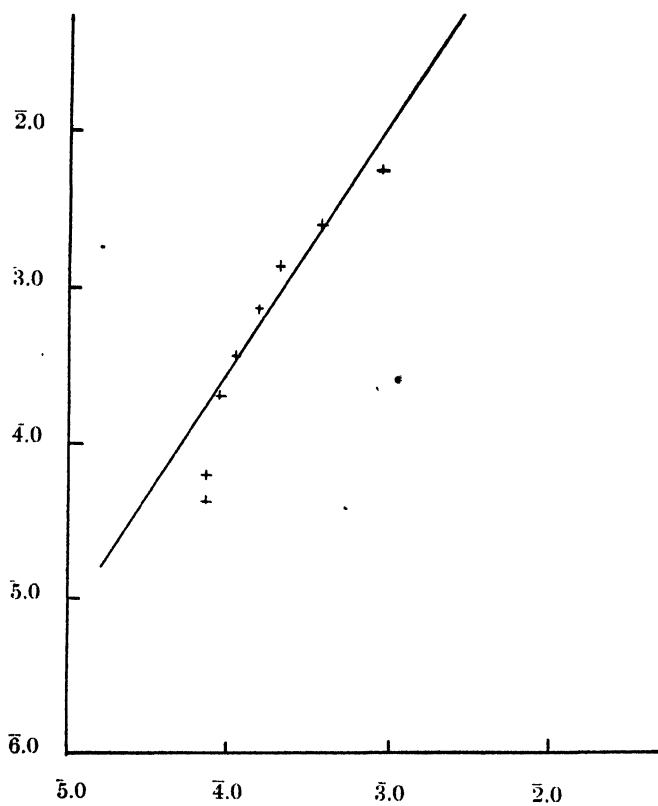


Figure 4.  
Phosphoric Acid and Calcium Silicate.  
(See Table 4)

TABLE 5

## LACTIC ACID AND CALCIUM SILICATE

Concentration	C <sub>H</sub> , H ion Lactic Acid	Log. H ion Lactic Acid	H ion Lactic Acid + Calcium Silicate	C <sub>a</sub> , Calcium Mols. per Liter	Log. Calcium Mols. per Liter
N 5	0.00561	3.74896	0.00577	0.00308	3.488551
N 25	0.00252	3.40140	0.000130	0.00201	3.303196
N 50	0.00169	3.22789	0.00000108	0.00123	3.089905
N 100	0.00114	3.05690	0.000000572.	0.000812	4.909556
N 250	0.000677	4.83059	0.000000141	0.000445	4.648360
N 500	0.000472	4.67394	0.0000000877	0.000208	4.318063
N 1,000	0.000270	4.43136	0.0000000719	0.000148	4.170262
N 2,000	0.000189	4.27646	0.0000000364	0.000099	5.995635
N 5,000	0.000112	4.04922	0.0000000395	0.000079	5.897627
N 10,000	0.0000547	5.73799	0.000000126	0.000074	5.869232

Constants for the equation  $\frac{C_H^x}{C_a} = K$ :

By graphical average:

$$x = 1.144$$

$$K = 14.86$$

Constants by calculation:

$$x = 1.118 \pm 0.0368 \quad C. v. = 25.9\%$$

$$K = 12.09 \pm 0.326 \quad C. v. = 12.01\%$$

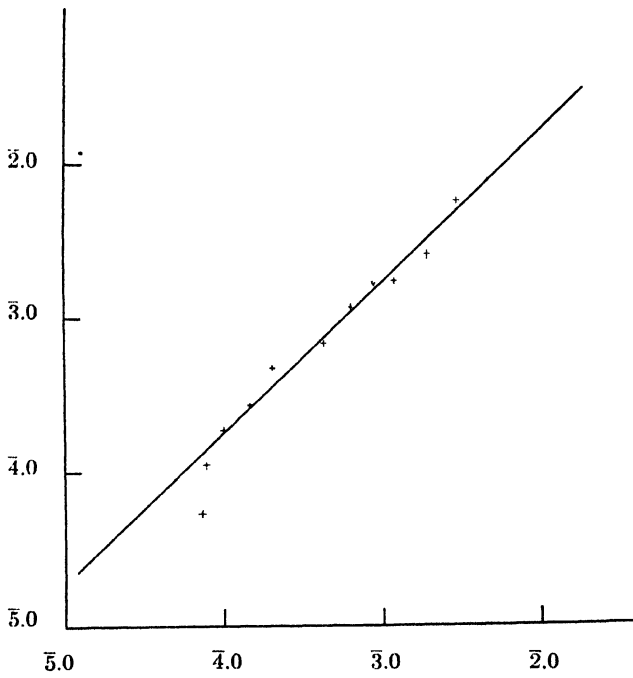


Figure 5.  
Lactic Acid and Calcium Silicate.  
(See Table 5)

TABLE 6  
FORMIC ACID AND CALCIUM SILICATE

Concentration	Ch. H ion Formic Acid	Log. H ion Formic Acid	H ion Formic Acid +Calcium Silicate	C <sub>a</sub> , Calcium Mols. per Liter	Log. Calcium Mols. per Liter
N 5	0.00518	$\bar{3}.71433$	0.00577	. . .	. . .
N 25	0.00233	$\bar{3}.36736$	0.0000635	. . .	. . .
N 50	0.00156	$\bar{3}.19312$	0.000000620	0.00119	$\bar{3}.075547$
N 100	0.0010	$\bar{3}.00000$	0.000000327*	0.000694	$\bar{4}.841359$
N 250	0.000762	$\bar{4}.88195$	0.0000000719	0.000365	$\bar{4}.563481$
N 500	0.000472	$\bar{4}.67394$	0.0000000522	0.000208	$\bar{4}.318063$
N 1,000	0.000343	$\bar{4}.53529$	0.0000000350	0.000138	$\bar{4}.139879$
N 2,000	0.000181	$\bar{4}.25768$	0.0000000276	0.000119	$\bar{4}.075547$
N 5,000	0.0000753	$\bar{5}.87679$	0.0000000245	0.000099	$\bar{5}.995635$
N 10,000	0.0000398	$\bar{5}.59988$	0.0000000719	0.0000248	$\bar{5}.394452$

Constants for the equation  $\frac{C_{II}^x}{C_u} = K$ :

$$x = 1.324$$

$$K = 335.5$$

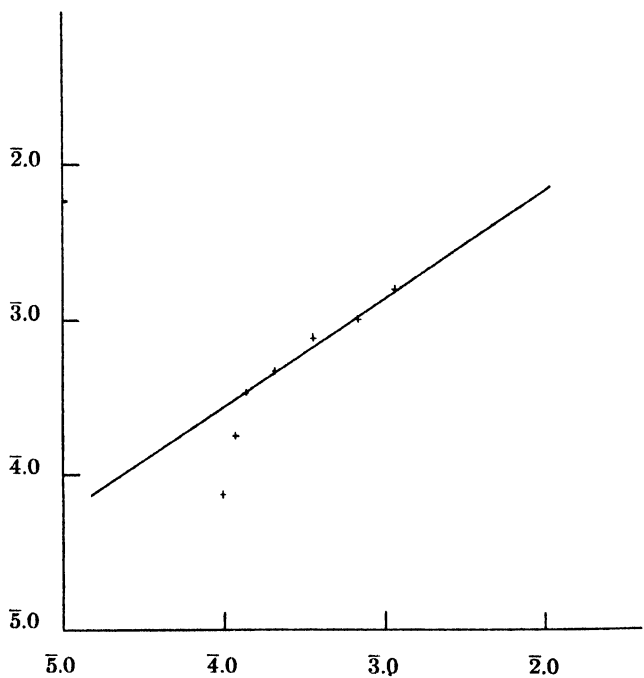


Figure 6.  
Formic Acid and Calcium Silicate.  
(See Table 6)

TABLE 7  
ACETIC ACID AND CALCIUM SILICATE

Concentration	C <sub>a</sub> , H ion Acetic Acid	Log. H ion Acetic Acid	H ion Acetic Acid + Calcium Silicate	C <sub>a</sub> , Calcium Mols. per Liter	Log. Calcium Mols. per Liter
N 5	0.00224	3.35025	0.000547	0.00285	3.454845
N 25	0.000860	4.93450	0.00000797	0.00250	3.397940
N 50	0.000677	4.83059	0.00000100	0.00126	3.100371
N 100	0.000454	4.65706	0.000000279	0.000742	4.870404
N 250	0.000387	4.58771	0.000000116	0.000347	4.540329
N 500	0.000213	4.32838	0.0000000350	0.000178	4.250420
N 1,000	0.000189	4.27646	0.0000000395	0.0000892	5.950365
N 2,000	0.000104	4.01703	0.0000000350	0.0000694	5.841359
N 5,000	0.0000884	5.94645	0.000000116	0.0000694	5.841359
N 10,000	0.0000414	5.61700	0.000000238	0.0000495	5.694605

Constants for the equation  $\frac{C_H^x}{C_a} = K$ :

$$x = 1.465$$

$$K = 941.2$$

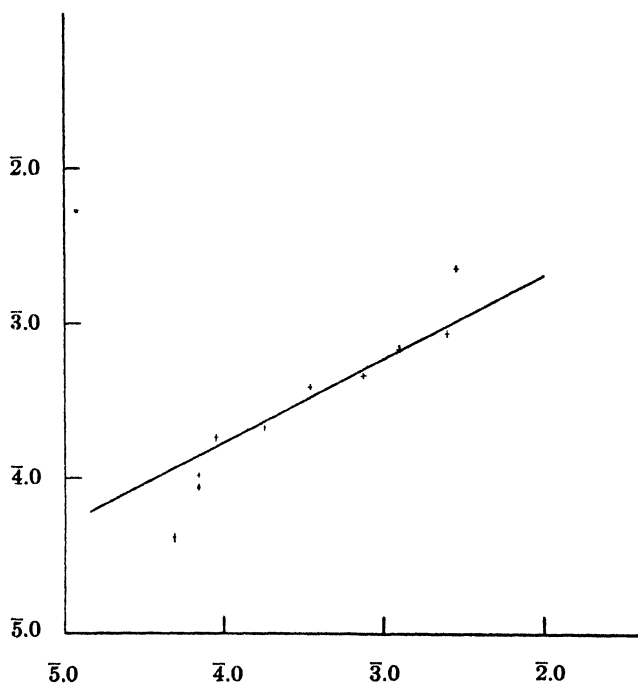


Figure 7.  
Acetic Acid and Calcium Silicate.  
(See Table 7)

TABLE 8  
HYDROCHLORIC ACID AND ORTHOCLASE

Concentration	C <sub>H</sub> , H ion Hydrochloric Acid	Log. H ion Hydrochloric Acid	H ion Hydrochloric Acid +Orthoclase	C <sub>a</sub> , Calcium Mols. per Liter	Log. Calcium Mols. per Liter
N 5	0.0882	2.94547	0.0615	0.00159	3.201397
N 25	0.0218	2.33846	0.0218	0.001449	3.159567
N 50	0.0135	2.13003	0.00836	0.001045	3.019116
N 100	0.00685	3.83569	0.00296	0.00094	4.973128
N 250	0.00321	3.50651	0.000554	0.000685	4.835691
N 500	0.00114	3.05690	0.0000784	0.000565	4.752048
N 1,000	0.000677	4.83059	0.000000935	0.00056	4.748188
N 2,000	0.000281	4.44871	0.00000110	0.00040	4.602060
N 5,000	0.0000326	5.51322	0.000000261	0.00038	4.579784
N 10,000	0.00000192	6.28330	0.00000010	0.000336	4.526339

Constants for Calcium:

$$x = 0.225$$

$$K = 0.000006310$$



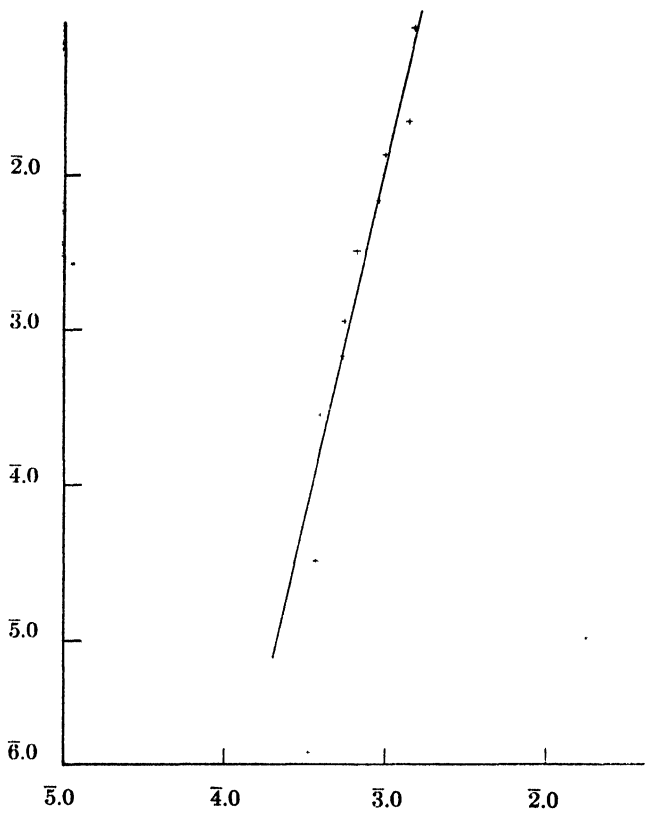


Figure 8.  
Hydrochloric Acid and Orthoclase.  
(See Table 8)

TABLE 9

## OXALIC ACID AND ORTHOCLASE

Concentration	Ch. H ion Oxalic Acid	Log. H ion Oxalic Acid	H ion Oxalic Acid +Orthoclase	C <sub>a</sub> , Calcium Mols. per Liter	Log. Calcium Mols. per Liter
N 5	0.0218	2.33846	0.0245	.....	.....
N 25	0.0110	2.04139	0.00905	.....	.....
N 50	0.00561	3.74896	0.00376	.....	.....
N 100	0.00347	3.54033	0.000650	.....	.....
N 250	0.00169	3.22789	0.00000564	.....	.....
N 500	0.00105	3.02119	0.000000735	.....	.....
N 1,000	0.000554	4.74351	0.000000438	.....	.....
N 2,000	0.000317	4.56937	0.000000294	.....	.....
N 5,000	0.000132	4.12057	0.00000294	.....	.....
N 10,000	0.0000785	5.89487	0.00000294	.....	.....

TABLE 10

## LACTIC ACID AND ORTHOCLASE

Concentration	Ch. H ion Lactic Acid	Log. H ion Lactic Acid	H ion Lactic Acid +Orthoclase	C <sub>a</sub> , Calcium Mols. per Liter	Log. Calcium Mols. per Liter
N 5	0.00561	3.74896	0.00441	0.001190	3.075547
N 25	0.00252	3.40140	0.00123	0.00094	4.973128
N 50	0.00169	3.22789	0.000650	0.00077	4.886491
N 100	0.00114	3.05690	0.000372	0.000495	4.694605
N 250	0.000677	4.83059	0.000181	0.00073	4.863323
N 500	0.000472	4.67394	0.0000414	0.000495	4.694605
N 1,000	0.000270	4.43136	0.00000322	0.000465	4.667453
N 2,000	0.000189	4.27646	0.00000217	0.000475	4.676694
N 5,000	0.000110	4.04139	0.000000282	0.000366	4.563481
N 10,000	0.0000547	5.73799	0.000000261	0.000238	4.376577

Constants for Calcium:

$$x = 0.324$$

$$K = 0.00002661$$

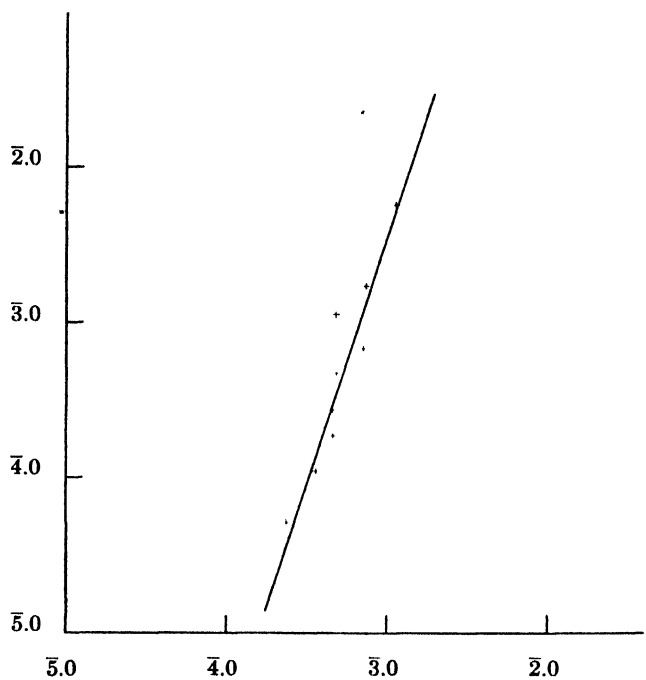


Figure 10.  
Lactic Acid and Orthoclase.  
(See Table 10)

TABLE 11  
FORMIC ACID AND ORTHOCLASE

Concentration	Ch. H ion Formic Acid	Log. H ion Formic Acid	H ion Formic Acid +Orthoclase	Ca. Calcium Mols. per Liter	Log. Calcium Mols. per Liter
N 5	0.00518	$\bar{3}.71433$	0.00296	0.000862	$\bar{4}.935507$
N 25	0.00233	$\bar{3}.36736$	0.000677	0.000812	$\bar{4}.909556$
N 50	0.00156	$\bar{3}.19312$	0.000270	0.000723	$\bar{4}.859138$
N 100	0.0010	$\bar{3}.00000$	0.0000957	0.000713	$\bar{4}.853090$
N 250	0.000762	$\bar{4}.88195$	0.0000153	0.000605	$\bar{4}.781755$
N 500	0.000472	$\bar{4}.67394$	0.00000579	0.000535	$\bar{4}.728354$
N 1,000	0.000343	$\bar{4}.53529$	0.000000197	0.000535	$\bar{4}.728354$
N 2,000	0.000181	$\bar{4}.25768$	0.000000197	0.000495	$\bar{4}.694605$
N 5,000	0.0000753	$\bar{5}.87679$	0.000000205	0.000475	$\bar{4}.676694$
N 10,000	0.0000398	$\bar{5}.59988$	0.0000000923	0.000426	$\bar{4}.629410$

Constants for Calcium:

$$x = 0.142$$

$$K = 0.000001427$$

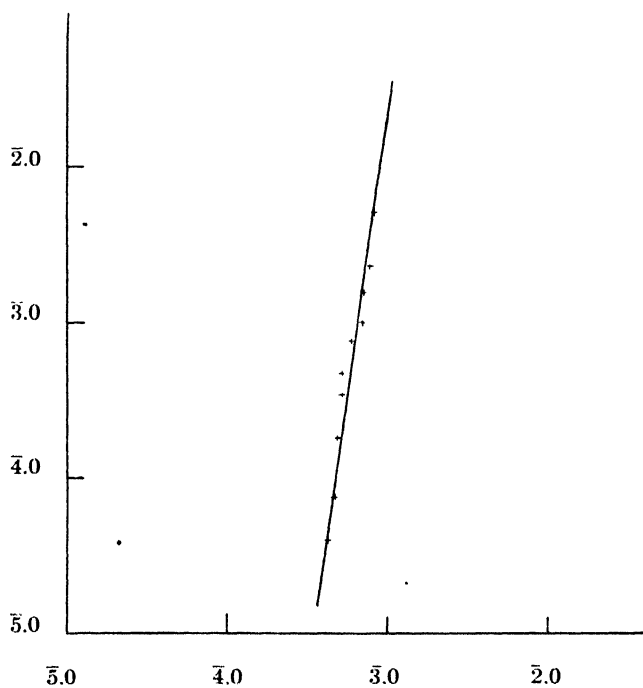


Figure 11.  
Formic Acid and Orthoclase.  
(See Table 11)

TABLE 12  
ACETIC ACID AND ORTHOCLASE.

Concentration	C <sub>H</sub> , H ion Acetic Acid	Log. H ion Acetic Acid	H ion Acetic Acid +Orthoclase	C <sub>Ca</sub> , Calcium Mols. per Liter	Log. Calcium Mols. per Liter
N 5	0.00224	3.35025	0.000472	0.000074	4.869232
N 25	0.000860	4.93450	0.000132	0.000058	4.763428
N 50	0.000677	4.83059	0.0000696	0.00062	4.792392
N 100	0.000454	4.65706	0.0000431	0.00065	4.812913
N 250	0.000387	4.58771	0.0000179	0.00051	4.707570
N 500	0.000213	4.32838	0.00000745	0.00048	4.681241
N 1,000	0.000189	4.27646	0.00000225	0.00043	4.633468
N 2,000	0.000104	4.01703	0.000000627	0.00039	4.591065
N 5,000	0.0000884	5.94645	0.000000438	0.00033	4.518514
N 10,000	0.0000414	5.61700	0.000000261	0.00029	4.462398

Constants for Calcium:

$$x = 0.235$$

$$K = 0.000006966$$

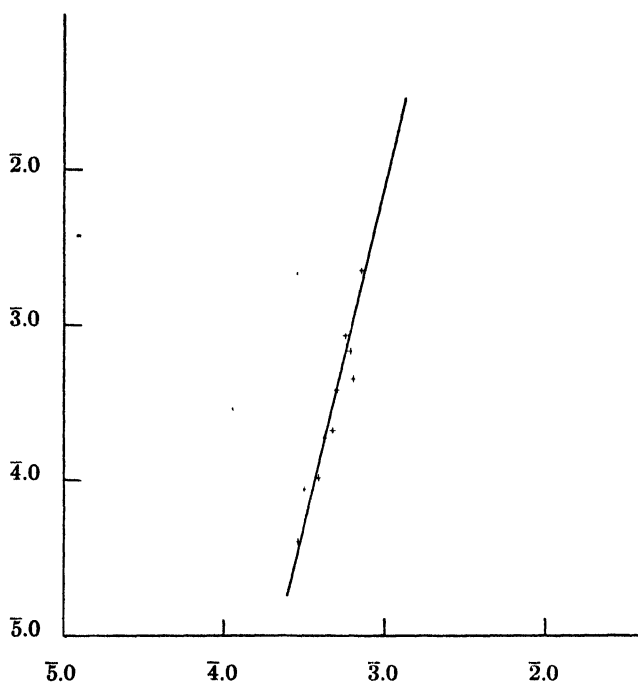


Figure 12.  
Acetic Acid and Orthoclase.  
(See Table 12)

TABLE 13.  
HYDROCHLORIC ACID AND BIOTITE.

Concentration HCl	Ca, H ion Hydrochloric Acid	Log. H ion Hydrochloric Acid	H ion Hydrochloric + Biotite	Ca, Calcium Mols. per Liter	Log. Calcium Mols. per Liter	Ca, Mag- nesium Mols. per Liter	Log. Mag- nesium Mols. per Liter	Ca, Potassium Mols. per Liter	Log. Potassium Mols. per Liter
N 5	0.0882	2.94547	0.0615	0.00385	3.585461	0.00457	3.659916	0.00158	3.198657
N 25	0.0218	2.33846	0.0146	0.00354	3.549003	0.00282	3.450249	0.00104	3.017033
N 50	0.0135	2.13003	0.00498	0.00351	3.545307	0.00201	3.303196	0.000856	4.932474
N 100	0.00685	3.83569	0.000512	0.00306	3.485721	0.00150	3.176091	0.000731	4.863917
N 250	0.00321	3.50651	0.000000863	0.00247	3.392697	0.000996	4.998259	0.000658	4.818226
N 500	0.00114	3.05690	0.0000000572	0.00191	3.281033	0.00066	4.819544	0.000597	4.775974
N 1,000	0.00677	4.83059	0.0000000912	0.00153	3.184691	0.00044	4.643453	0.000535	4.728354
N 2,000	0.00281	4.44871	0.00000000350	0.00117	3.068186	0.00031	4.491352	0.000383	4.583199
N 5,000	0.000326	5.51322	0.00000000270	0.00111	3.045323	0.00026	4.414973	0.000366	4.563481
N 10,000	0.0000192	6.28330	0.00000000217	0.00081	4.908485	0.00028	4.447158	0.00035	4.544068

Constants for Calcium:

$$x = 0.288$$

$$K = 0.000006054$$

Constants for Magnesium:

$$x = 0.490$$

$$K = 0.0004667$$

Constants for Potassium:

$$x = 0.219$$

$$K = 0.000006746$$



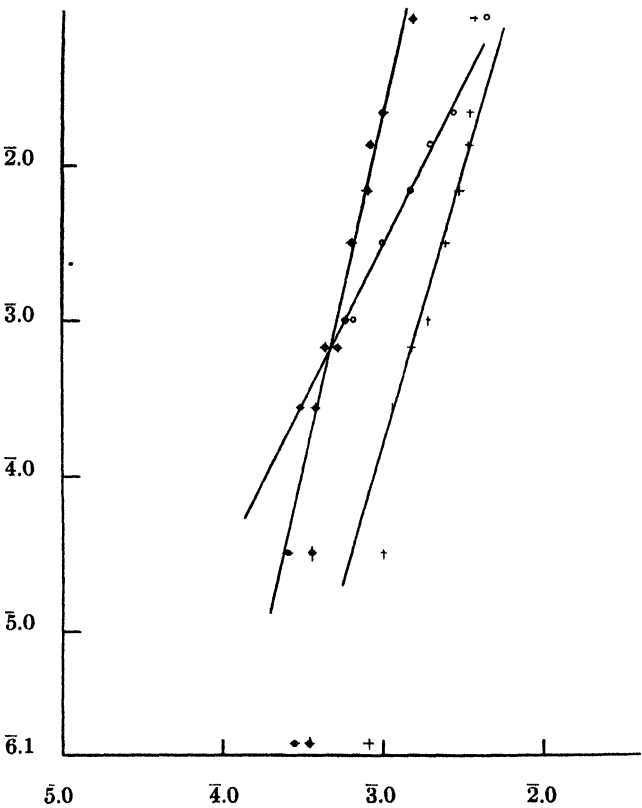


Figure 13.  
Hydrochloric Acid and Biotite.  
(See Table 13)

TABLE 14.  
SULFURIC ACID AND BIOTITE.

Concentration	Ch <sub>2</sub> H ion Sulfuric Acid	Log. H ion Sulfuric Acid	H ion Sulfuric Acid + Biotite	C <sub>a</sub> , Calcium Mols. per Liter	Log. Calcium Mols. per Liter	C <sub>a</sub> , Mag- nesium Mols. per Liter	Log. Mag- nesium Mols. per Liter	C <sub>a</sub> , Potassium Mols. per Liter	Log. Potassium Mols. per Liter
N 5	0.0567	2.75358	0.0313	0.00393	3.594393	0.00629	3.798651	0.00212	3.326336
N 25	0.0186	2.26951	0.00836	0.00326	3.513218	0.002705	3.432167	0.00133	3.123852
N 50	0.0130	2.11394	0.00472	0.00308	3.488551	0.001835	3.263636	0.00162	3.209515
N 100	0.00632	3.80072	0.00343	0.00291	3.463893	0.00137	3.136721	0.00145	3.161368
N 250	0.00296	3.47129	0.00000967	0.00252	3.301401	0.000745	4.872156	0.00104	3.017033
N 500	0.00162	3.20952	0.00000004	0.00204	3.309630	0.000585	4.767156	0.00090	4.954243
N 1,000	0.000931	4.96895	0.00000000719	0.00182	3.260071	0.000378	4.577492	0.000694	4.841359
N 2,000	0.000600	4.77815	0.0000000302	0.00144	3.158362	0.000324	4.510545	0.000468	4.670246
N 5,000	0.000259	4.41330	0.00000000217	0.00106	3.025306	0.000252	4.401401	0.000432	4.635484
N 10,000	0.000174	4.24055	0.0000000002	0.00085	4.929419	0.000288	4.459392	0.000395	4.596597

Constants for Calcium:

$$x = 0.188$$

$$K = 0.000001071$$

Constants for Magnesium:

$$x = 0.611$$

$$K = 0.004571$$

Constants for Potassium:

$$x = 0.316$$

$$K = 0.00002379$$

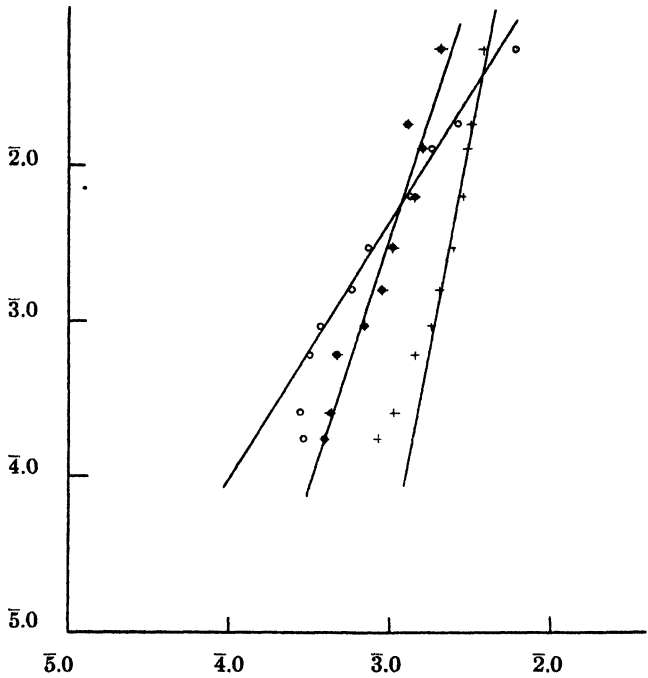


Figure 14.  
Sulfuric Acid and Biotite.  
(See Table 14)

TABLE 15  
OXALIC ACID AND BIOTITE

Concentration	C <sub>a</sub> , H ion Oxalic Acid	Log. H ion Oxalic Acid	H ion Oxalic Acid + Biotite	C <sub>a</sub> , Calcium Mols per Liter	Log. Calcium Mols. per Liter	C <sub>a</sub> , Mag- nesium Mols. per Liter	Log. Mag- nesium Mols. per Liter	C <sub>a</sub> , Potassium Mols. per Liter	Log. Potassium Mols. per Liter
N 5	0.0218	2.33846	0.0277			0.00106	3.025306	....	.....
N 25	0.0110	2.04139	0.00347			0.001375	3.138303	....	.....
N 50	0.00561	3.74896	0.000600			0.001735	3.239299	....	.....
N 100	0.00347	3.54033	0.000120			0.000995	4.995635	....	.....
N 250	0.00169	3.22789	0.0000010			0.00072	4.857332	....	.....
N 500	0.00105	3.02119	0.000000257			0.00052	4.792392	....	.....
N 1,000	0.000554	4.74351	0.000000107			0.00064	4.806180	....	.....
N 2,000	0.000317	4.50106	0.0000000428			0.00069	4.770852	....	.....
N 5,000	0.000132	4.12057	0.0000000035			0.00038	4.579784	....	.....
N 10,000	0.0000785	5.89487	0.00000000637			0.00033	4.518514	....	.....

Constants for Magnesium:

$$x = 0.291$$

$$K = 0.00001579$$

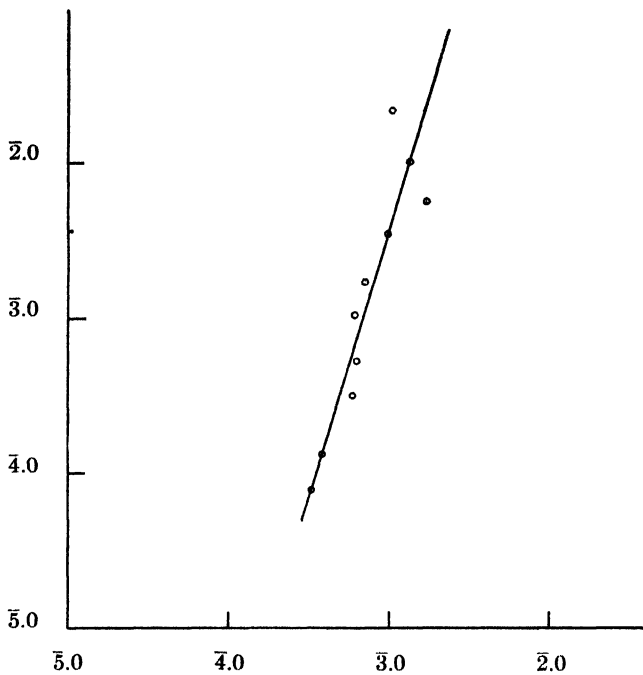


Figure 15.  
Oxalic Acid and Biotite.  
(See Table 15)

TABLE 16

## PHOSPHORIC ACID AND BIOTITE

Concentration	Ch. H ion Phosphoric Acid	Log. H ion Phosphoric Acid	H ion Phosphoric + Biotite	C <sub>a</sub> , Calcium Mols. per Liter	Log. Calcium Mols. per Liter	C <sub>a</sub> , Mag- nesium Mols. per Liter	Log. Mag- nesium Mols. per Liter	C <sub>p</sub> , Potassium Mols. per Liter	Log. Potassium Mols. per Liter
N 5	0.0125	2.09691	0.0115	0.00342	3.534926	0.00687	3.836957	0.00155	3.190332
N 25	0.00632	3.80072	0.00233	0.00324	3.510545	0.00368	3.565848	0.00134	3.127105
N 50	0.00424	3.62737	0.000230	0.00300	3.477121	0.00339	3.530200	0.00117	3.063186
N 100	0.00273	3.43616	0.00000208	0.00264	3.421604	0.00203	3.307496	0.000834	3.921166
N 250	0.00144	3.15836	0.000000144	0.00103	3.367356	0.00103	3.012837	0.000238	3.376577
N 500	0.000762	3.88195	0.0000000416	0.00168	3.225309	0.00063	4.799341	0.000255	4.406540
N 1,000	0.000372	4.57054	0.0000000147	0.00132	3.120574	0.000503	4.701568	0.000263	4.419956
N 2,000	0.000213	4.32838	0.00000000663	0.00105	3.021189	0.000332	4.521138	0.000255	4.406540
N 5,000	0.0000642	5.80754	0.00000000379	0.00090	4.556303	0.00036	4.556303	0.000246	4.390935
N 10,000	0.0000431	5.63448	0.000000000778	0.00072	4.863323	0.000512	4.709270	0.000304	4.482874

Constants for Calcium:

$$x = 0.290$$

$$K = 0.000005930$$

Constants for Magnesium:

$$x = 0.714$$

$$K = 0.009398$$

Constants for Potassium:

$$x = 0.392$$

$$K = 0.00008882$$

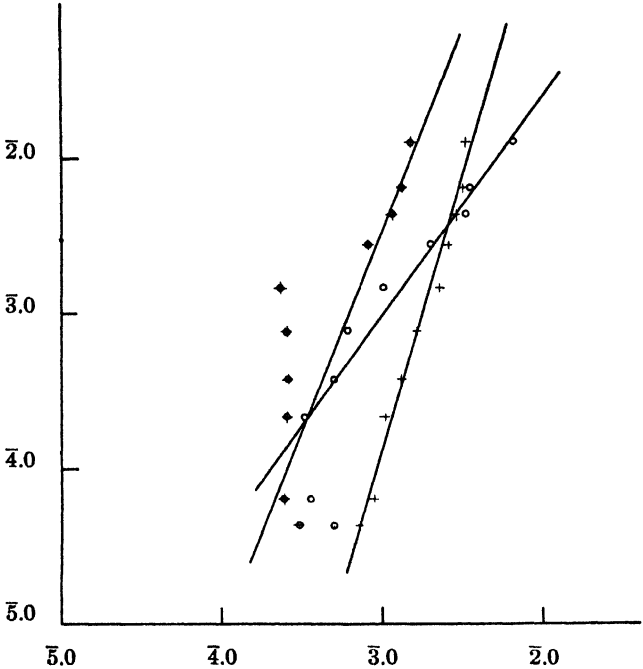


TABLE 17  
LACTIC ACID AND BIOTITE

Concentration	Ca, H ion Lactic Acid	Log. H ion Lactic Acid	H ion Lactic Acid + Biotite	Ca, Calcium Mols. per Liter	Log. Calcium Mols. per Liter	Ca, Mag- nesium Mols. per Liter	Log. Mag- nesium Mols. per Liter	Ca, Potassium Mols. per Liter	Log. Potassium Mols. per Liter
N 5	0.00561	3.74896	0.00233	0.00332	3.521138	0.00254	3.404834	0.000349	4.542825
N 25	0.00252	3.40140	0.000472	0.00314	3.496930	0.00190	3.278754	0.000505	4.703291
N 50	0.00169	3.22789	0.000181	0.00293	3.466868	0.00153	3.184691	0.000427	4.630428
N 100	0.00114	3.05690	0.0000398	0.00260	3.414973	0.00133	3.123852	0.000370	4.568202
N 250	0.000677	4.83059	0.00000735	0.00230	3.361728	0.000845	4.926857	0.000218	4.338456
N 500	0.000472	4.67394	0.000000302	0.00172	3.235528	0.00053	4.724276	0.000185	4.267172
N 1,000	0.000270	4.43136	0.0000000778	0.00131	3.117271	0.00046	4.662758	0.000210	4.322219
N 2,000	0.000189	4.27646	0.00000000350	0.00098	4.991226	0.00033	4.518514	0.000156	4.193125
N 5,000	0.000112	4.04922	0.00000000217	0.00087	4.939519	0.00017	4.230449	0.000148	4.170262
N 10,000	0.0000547	5.73799	0.00000000384	0.00062	4.792392	0.000144	4.158362	0.000210	4.322219

Constants for Calcium:

$$x = 0.443$$

$$K = 0.00005566$$

Constants for Magnesium:

$$x = 0.743$$

$$K = 0.04373$$

Constants for Potassium:

$$x = 0.396$$

$$K = 0.000166$$



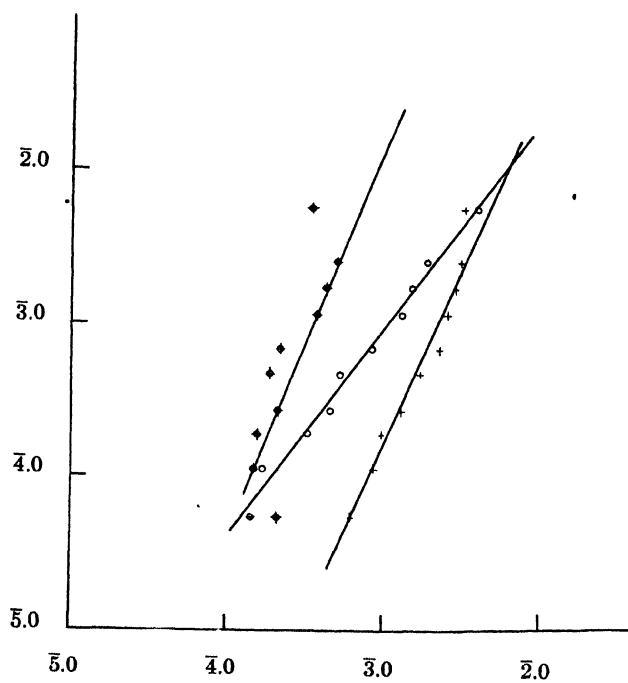


Figure 17.  
Lactic Acid and Biotite.  
(See Table 17)

TABLE 18  
FORMIC ACID AND BIOTITE

Concentration	Ch. H ion Formic Acid	Log. H ion Formic Acid	H ion Formic Acid +Biotite	C <sub>a</sub> Calcium Mols. per Liter	Log. Calcium Mols. per Liter	C <sub>m</sub> Mag- nesium Mols. per Liter	Log. Mag- nesium Mols. per Liter	C <sub>p</sub> Potassium Mols. per Liter	Log. Potassium Mols. per Liter
N 5	0.00518	3.71433	0.00252	0.00310	3.491362	0.00149	3.173186	0.000888	4.948413
N 25	0.00233	3.36736	0.000587	0.00306	3.485721	0.00127	3.103804	0.000847	4.927883
N 50	0.00156	3.19312	0.000249	0.00285	3.454845	.....	.....	0.000744	4.871573
N 100	0.0010	3.0000	0.0000753	0.00252	3.401401	0.000503	4.701568	0.000436	4.639486
N 250	0.000763	4.88195	0.00000935	0.00239	3.378398	0.000314	4.496930	0.000325	4.511883
N 500	0.000472	4.67394	0.000000778	0.00171	3.232996	0.00027	4.431364	0.000308	4.498551
N 1,000	0.000343	4.53529	0.0000000411	0.00131	3.117271	0.00038	4.579784	0.000275	4.439333
N 2,000	0.000181	4.25768	0.0000000276	0.00097	4.986772	0.000234	4.369216	0.000279	4.445604
N 5,000	0.0000753	5.87679	0.0000000134	0.00089	4.949390	0.00017	4.230449	0.000271	4.432969
N 10,000	0.0000398	5.59988	0.0000000315	0.00085	4.767156	0.00016	4.204120	0.000226	4.354108

Constants for Calcium:

$$x = 0.447$$

$$K = 0.00005790$$

Constants for Magnesium:

$$x = 0.529$$

$$K = 0.0007431$$

Constants for Potassium:

$$x = 0.272$$

$$K = 0.00001808$$

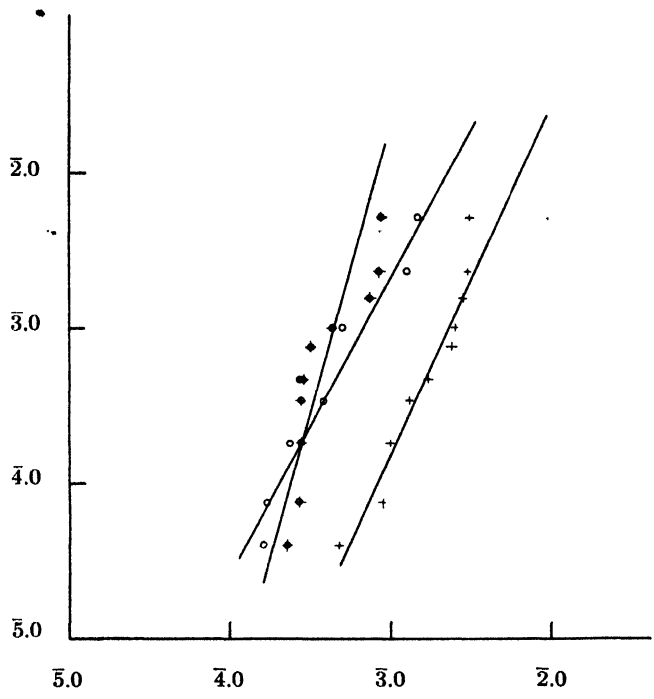


Figure 18.  
Formic Acid and Biotite.  
(See Table 18)

TABLE 19

## ACETIC ACID AND BIOTITE

Concentration	Ch. H ion Acetic Acid	Log. H ion Acetic Acid	H ion Acetic Acid +Biotite	C <sub>a</sub> Calcium Mols. per Liter	Log. Calcium Mols. per Liter	C <sub>a</sub> Mag- nesium Mols. per Liter	Log. Mag- nesium Mols. per Liter	C <sub>a</sub> Potassium Mols. per Liter	Log. Potassium Mols. per Liter
N 5	0.00224	5.35025	0.000343	0.00295	5.469822	0.00196	5.292256	0.000687	4.836957
N 25	0.000860	4.93450	....	...	...	...	...	...	....
N 50	0.000677	4.83059	0.0000313	0.00268	5.428135	0.00164	5.214844	0.000530	4.724276
N 100	0.000454	4.65706	....	...	...	...	...	...	....
N 250	0.000387	4.58771	0.00000579	0.00235	5.371068	0.00128	5.107210	0.000271	4.432969
N 500	0.000313	4.32838	...	...	...	...	...	...	...
N 1,000	0.000189	4.27646	0.00000000445	0.00145	5.161368	0.00150	5.176091	0.000263	4.419956
N 2,000	0.000104	4.01703	.....	...	...	...	...	...	...
N 5,000	0.0000884	5.94645	0.00000000245	0.00079	4.897627	0.000845	4.926857	0.000255	4.406540
N 10,000	0.0000414	5.61700	0.00000000663	0.00058	4.763428	0.000585	4.767156	0.000271	4.432969

Constants for Calcium:

$$x = 0.603$$

$$K = 0.0004426$$

Constants for Magnesium:

$$x = 0.308$$

$$K = 0.00008395$$

Constants for Potassium:

$$x = 0.310$$

$$K = 0.00002812$$

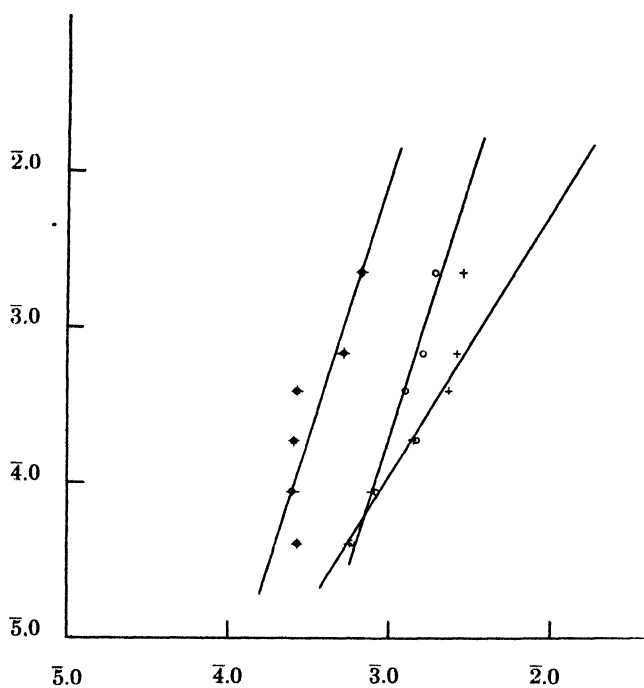


Figure 19.  
Acetic Acid and Biotite.  
(See Table 19)

TABLE 20  
HYDROCHLORIC ACID AND GRANITE

Concentration	C <sub>H</sub> , H ion Hydrochloric Acid	Log. H ion Hydrochloric Acid	H ion Hydrochloric +Granite	C <sub>H</sub> , Calcium Mols. per Liter	Log. Calcium Mols. per Liter	C <sub>H</sub> , Calcium Mols. per Liter	Log. Calcium Mols. per Liter
N 5	0.0882	2.94547	0.0567	0.00595	3.774517	0.00139	3.143015
N 25	0.0218	2.33846	0.0165	0.00536	3.729165	0.00135	3.130334
N 50	0.0135	2.13003	0.00607	0.00432	3.635484	0.00116	3.064458
N 100	0.0085	3.83569	0.00144	0.00333	3.522444	0.00103	3.012837
N 250	0.00321	3.50651	0.000228	0.00167	3.222716	0.000832	4.920123
N 500	0.00114	3.05690	0.0000216	0.000782	4.693207	0.000654	4.815578
N 1,000	0.00677	4.83059	0.0000192	0.000287	4.457882	0.000605	4.781755
N 2,000	0.00281	4.44871	0.00000753	0.000129	4.110590	0.000535	4.728354
N 5,000	0.000326	5.51322	0.000000852	0.000119	4.075547	0.000356	4.551450
N 10,000	0.0000192	6.28330	0.000000653	0.000119	4.075547	0.000158	4.191657

Constants for Calcium:

$$x = 0.204$$

$$K = 0.000004056$$

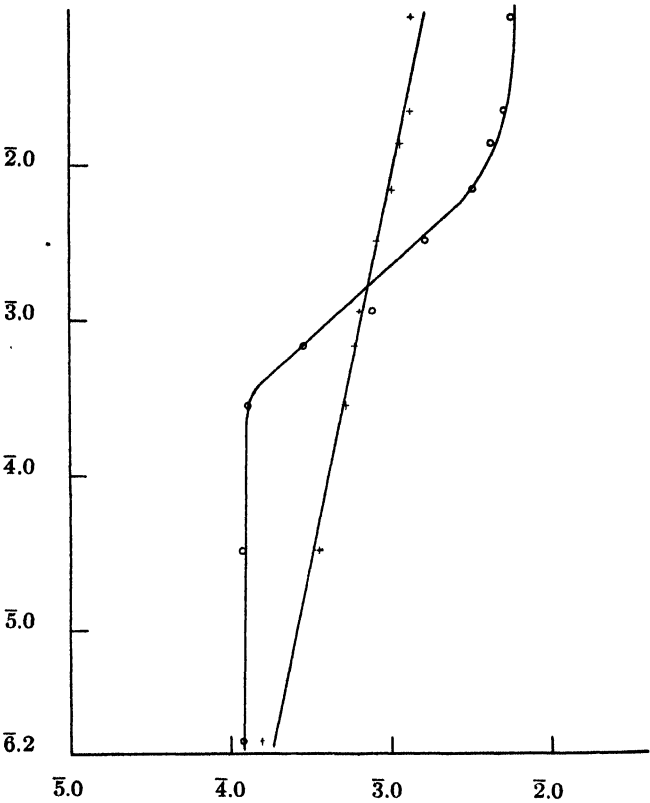


Figure 20.  
Hydrochloric Acid and Granite.  
(See Table 20)

TABLE 21

SULFURIC ACID AND GRANITE

Concentration	$C_{H^+}$ H ion Sulfuric Acid	Log. H ion Sulfuric Acid	H ion Sulfuric Acid +Granite	$C_{Fe}$ Iron Mols. per Liter	Log. Iron Mols. per Liter	$C_{Ca}$ Calcium Mols. per Liter	Log. Calcium Mols. per Liter
N 5	0.0567	2.75358	0.0300	. . . .	. . . .	0.00135	3.130334
N 25	0.0186	2.26951	0.0115	0.00385	3.585461	0.00127	3.103804
N 50	0.0130	3.11394	0.00539	0.00321	3.506505	0.00127	3.103804
N 100	0.00632	3.80072	0.00183	0.00204	3.309630	0.00101	3.004321
N 250	0.00296	3.47129	0.000117	0.00103	3.012837	0.000901	4.954725
N 500	0.00162	3.70952	0.0000349	0.000455	4.658011	0.000352	4.930440
N 1,000	0.000931	4.96895	0.00000119	0.000192	4.296665	0.000792	4.898725
N 2,000	0.000600	4.77815	0.000000197	0.000109	4.037426	0.000783	4.293762
N 5,000	0.000259	4.41330	0.0000000384	0.000109	4.037426	0.000576	4.759668
N 10,000	0.000174	4.24055	0.0000000421	0.000059	5.770852	0.000338	4.528917

Constants for Calcium:

$x = 0.145$   
 $K = 0.000001328$



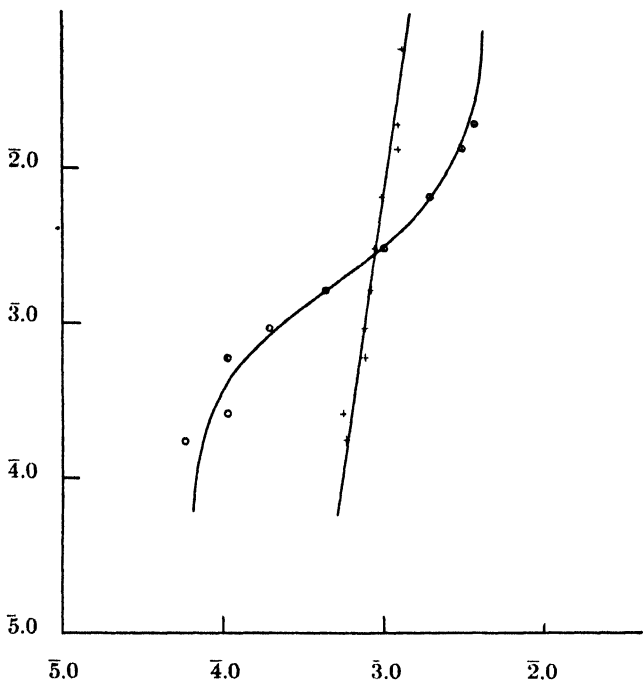


Figure 21.  
Sulfuric Acid and Granite.  
(See Table 21)

TABLE 22

## OXALIC ACID AND GRANITE

Concentration	Ch. H ion Oxalic Acid	Log. H ion Oxalic Acid	H ion Oxalic Acid +Granite	C <sub>a</sub> , Iron Mols. per Liter	Log. Iron Mols. per Liter
N 5	0.0213	2.33846	0.0255	.....	.....
N 25	0.0110	2.04139	0.00772	.....	.....
N 50	0.00561	3.74896	0.00226	.....	.....
N 100	0.00347	3.54033	0.00000129	.....	.....
N 250	0.00169	3.22789	0.0000141	.....	.....
N 500	0.00105	3.02119	0.00000254	.....	.....
N 1,000	0.000554	4.74351	0.000000863	.....	.....
N 2,000	0.000317	4.50106	0.000000421	.....	.....
N 5,000	0.000132	4.12057	0.000000241	.....	.....
N 10,000	0.0000785	5.89487	0.000000261	.....	.....

TABLE 23

## PHOSPHORIC ACID AND GRANITE

Concen- tration	Ch. H ion Phosphoric Acid	Log. H ion Phosphoric Acid	H ion Phosphoric Acid +Granite	C <sub>a</sub> , Iron Mols. per Liter	Log. Iron Mols. per Liter	C <sub>a</sub> , Calcium Mols. per Liter	Log. Calci- um Mols. per Liter
N 5	0.0125	2.09691	0.001	0.00496	3.695482	0.00174	3.232996
N 25	0.00632	3.80072	0.00296	0.00410	3.612784	0.000119	4.075547
N 50	0.00424	3.62737	0.0001	0.00351	3.545307	0.000178	4.250420
N 100	0.00273	3.43616	0.0000486	0.00716	3.334454	0.000515	4.711807
N 250	0.00144	3.15836	0.00000461	0.000675	4.829304	0.000505	4.703291
N 500	0.000762	4.88195	0.000000232	0.000308	4.488551	0.000416	4.619093
N 1,000	0.000372	4.57054	0.000000117	0.000188	4.274158	0.000436	4.639486
N 2,000	0.000213	4.32838	0.0000000787	0.000159	4.201397	0.000386	4.586587
N 5,000	0.0000642	5.80754	0.0000000961	0.000148	4.170262	0.000297	4.472756
N 10,000	0.0000431	5.63448	0.000000222	0.000119	4.075547	0.000376	4.575188

Constants for Calcium:

x = 0.136

K = 0.000001987

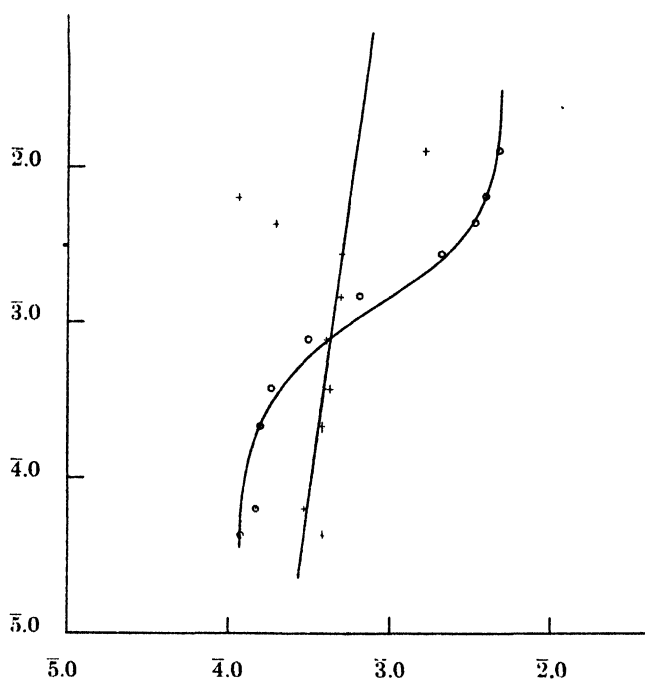


Figure 23.  
Phosphoric Acid and Granite.  
(See Table 23)

TABLE 24

## LACTIC ACID AND GRANITE

Concentration	Ch. H ion Lactic Acid	Log H ion Lactic Acid	H on Lactic Acid + Granite	C <sub>a</sub> , Iron Mols. per Liter	Log. Iron Mols. per Liter	C <sub>a</sub> , Calcium Mols. per Liter	Log. Calcium Mols. per Liter
N 5	0.00561	3.74896	0.00321	0.00492	3.691965	0.00144	3.158362
N 25	0.00252	3.40140	0.00677	0.00402	3.604226	0.00118	3.071882
N 50	0.00169	3.22789	0.00317	0.00302	3.480007	0.00100	3.000000
N 100	0.00114	3.05690	0.00167	0.00184	3.264818	0.00101	3.004321
N 250	0.000677	4.83059	0.000339	0.000555	4.744293	0.000783	4.893762
N 500	0.000472	4.67394	0.0000564	0.000238	4.376577	0.000783	4.893762
N 1,000	0.000270	4.43136	0.0000309	0.00009	5.954243	0.000634	4.802089
N 2,000	0.000189	4.27646	0.0000216	0.0000694	5.841359	0.000733	4.865104
N 5,000	0.000112	4.04922	0.0000335	0.000119	4.728354	0.000535	4.728354
N 10,000	0.0000547	5.73799	0.0000753	0.0000694	5.841359	0.000455	4.658011

Constants for Calcium:

$$x = 0.246$$

$$K = 0.000005781$$

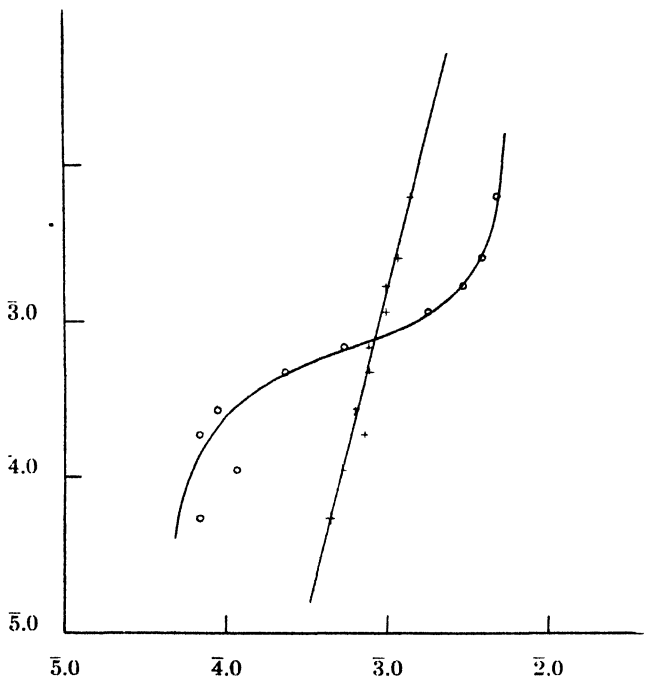


Figure 24.  
Lactic Acid and Granite.  
(See Table 24)

TABLE 25

## FORMIC ACID AND GRANITE

Concentration	Ca, H ion Formic Acid	Log. H ion Formic Acid	H ion Formic Acid +Granite	Ca, Iron Mols. per Liter	Log. Iron Mols. per Liter	Ca, Calcium Mols. per Liter	Log. Calcium Mols. per Liter
N 5	0.00518	3.71433	0.00233	0.00474	3.675778	0.00135	3.130334
N 25	0.00233	3.36736	0.000554	0.00456	3.658965	0.00108	3.039424
N 50	0.00156	3.19312	0.000249	0.00387	3.587711	0.00101	3.004321
N 100	0.0010	3.00000	0.000137	0.00226	3.354108	0.00094	4.973128
N 250	0.000762	4.88195	0.0000228	0.000891	4.949390	0.00088	4.944483
N 500	0.000472	4.67394	0.00000129	0.000802	4.904174	0.000772	4.887617
N 1,000	0.000343	4.53529	0.000000967	0.000208	4.318063	0.000634	4.802089
N 2,000	0.000181	4.25768	0.000000513	0.00009	5.954243	0.000575	4.759668
N 5,000	0.0000753	5.87679	0.0000000787	0.000079	5.897627	0.000485	4.685742
N 10,000	0.0000398	5.59988	0.0000000117	0.00009	5.954243	0.000366	4.563481

Constants for Calcium:

$$x = 0.263$$

$$K = 0.000007799$$

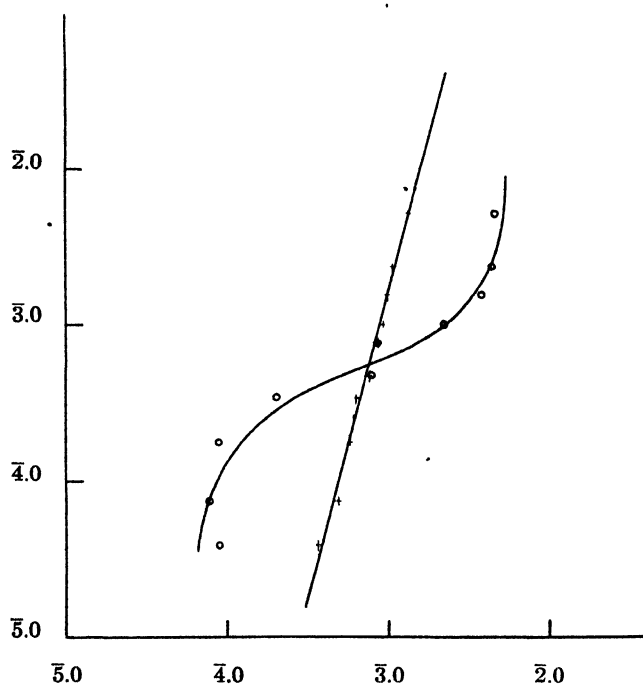


Figure 25.  
Formic Acid and Granite.  
(See Table 25)

TABLE 26

## ACETIC ACID AND GRANITE

Concentration	C <sub>h</sub> H ion Acetic Acid	Log H ion Acetic Acid	H ion Acetic Acid + Granite	C <sub>a</sub> Iron Mols. per Liter	Log Iron Mols. per Liter	C <sub>a</sub> Calcium Mols. per Liter	Log Calcium Mols. per Liter
N 5	0.00224	3.35025	0.000343	0.00377	3.576341	0.00113	3.053078
N 25	0.000860	4.93450	0.0000596	0.00356	3.551450	0.00105	3.021189
N 50	0.000677	4.83059	0.0000415	0.00215	3.332438	0.00102	3.008600
N 100	0.000454	4.65706	0.0000228	0.00121	3.082785	0.000970	4.986772
N 250	0.000387	4.58771	0.00000687	0.000625	4.795880	0.000792	4.898725
N 500	0.000213	4.32838	0.0000110	0.000455	4.658011	0.000782	4.893207
N 1,000	0.000189	4.27646	0.000000863	0.000178	4.250420	0.000734	4.865696
N 2,000	0.000104	4.01703	0.000000653	0.000089	5.949390	0.000525	4.720159
N 5,000	0.0000884	5.94645	0.000000302	0.00009	5.954243	0.000584	4.766413
N 10,000	0.0000414	5.61700	0.0000000646	0.000109	4.037426	0.000495	4.694605

Constants for Calcium:

$$x = 0.232$$

$$K = 0.000004113$$



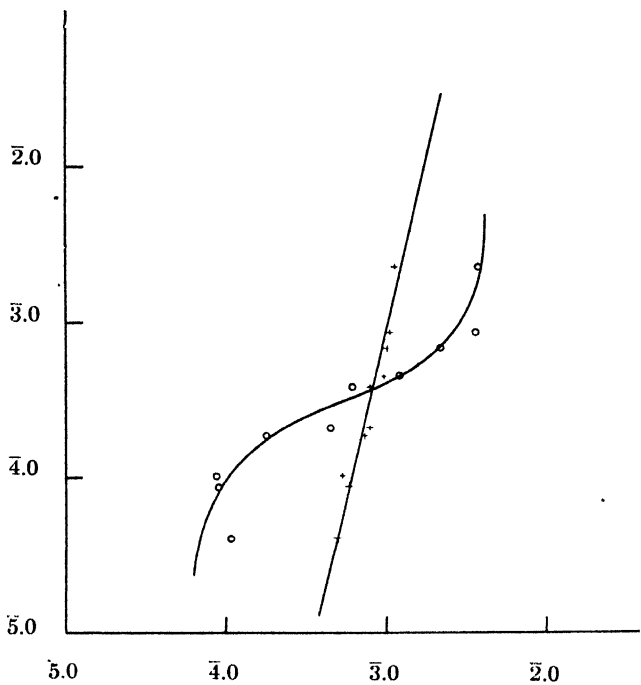


Figure 26.  
Acetic Acid and Granite.  
(See Table 26)

TABLE 27.

## ORTHOCLASE AND AZOTOBACTER.

Time Days	C <sub>H</sub> , H ion Dextrose +Azotobacter	Log. H ion Dextrose +Azotobacter	H ion Orthoclase +Azotobacter	C <sub>a</sub> , Calcium Mols. per Liter	Log. Calcium Mols. per Liter
0	0.00000282	$\bar{6}.450249$	0.000000962	0.000238	$\bar{4}.376577$
1	0.00000261	$\bar{6}.416641$	0.000000787	0.000675	$\bar{4}.829304$
2	0.00000331	$\bar{6}.519828$	0.00000161	0.000455	$\bar{4}.658011$
3	0.00000359	$\bar{6}.555094$	0.000000887	0.000575	$\bar{4}.774517$
5	0.00000421	$\bar{6}.624282$	0.000000887	0.000852	$\bar{4}.930440$
7	0.00000534	$\bar{6}.727541$	0.00000197	0.00107	$\bar{3}.029384$
9	0.00000627	$\bar{6}.797268$	0.00000251	0.00404	$\bar{3}.606381$
11	0.00000935	$\bar{6}.970812$	0.00000331	0.00198	$\bar{3}.296665$
16	0.0000177	$\bar{5}.247973$	0.00000389	0.00119	$\bar{3}.075547$

Constants for Calcium:

$$x = 1.804$$

$$K = 19.77$$

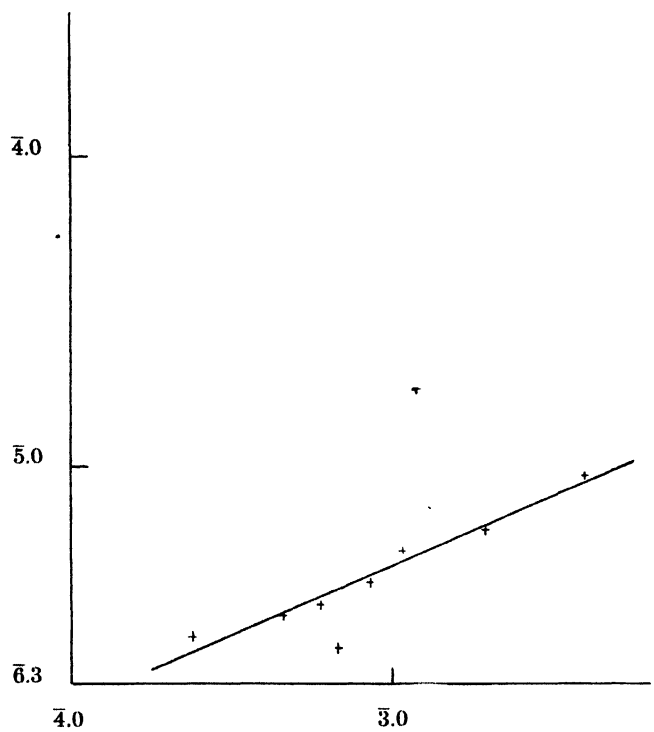


Figure 27.  
Orthoclase and Azotobacter.  
(See Table 27)

TABLE 28  
BIOTITE AND AZOTOBACTER

Time Days	Ch. H ion Dextrose+ Azotobacter	Log. H ion Dextrose+ Azotobacter	H ion Biotite+ Azotobacter	Ca, Calcium Mols. per Liter	Log. Calcium Mols. per Liter	Ca, Mag- nesium Mols. per Liter	Log. Mag- nesium Mols. per Liter	Ca, Potassium Mols. per Liter	Log. Potassium Mols. per Liter
0	0.00000282	6.450249	0.00000040	0.001605	3.205475	0.000862	4.935507	0.00079	4.897627
1	0.00000261	6.416641	0.000000384	0.00200	3.301030	0.001005	3.002166	0.000435	4.638489
2	0.00000331	6.519828	0.0000004	0.00220	3.342423	0.000628	4.797960	0.00036	4.556303
3	0.00000359	5.555094	0.00000034	0.00242	3.383815	0.000503	4.701568	0.000445	4.648360
5	0.00000421	6.624282	0.000000596	0.00244	3.387390	0.000484	4.684854	0.00042	4.623249
7	0.00000534	6.727541	0.00000132	0.00293	3.466868	0.000556	4.745075	0.000715	4.854306
9	0.00000627	6.797268	0.00000197	0.00275	3.439333	0.000807	4.906874	0.00069	4.838849
11	0.00000935	6.970812	0.00000282	0.00258	3.411620	0.001401	3.146438	0.000585	4.767156
16	0.0000177	5.247973	0.00000205	0.00258	3.411620	0.000000		0.000765	4.883661

Constants for Calcium:

$$x = 0.374$$

$$K = 0.000002198$$

Constants for Magnesium:

$$x = 1.312$$

$$K = 0.2388$$

Constants for Potassium:

$$x = 0.422$$

$$K = 0.00001963$$



TABLE 29  
GRANITE AND AZOTOBACTER

Time Days	C <sub>h</sub> , H ion Dextrose + Azotobacter	Log. H ion Dextrose + Azotobacter	H ion Granite + Azotobacter	C <sub>a</sub> , Calcium Mols. per Liter	Log. Calcium Mols per Liter	C <sub>h</sub> , Iron Mols. per Liter	Log. Iron Mols. per Liter
0	0.00000282	6.450249	0.000000887	0.000495	4.694605	0.000099	5.995635
1	0.00000261	6.416641	0.000000727	0.000792	4.898725	0.000376	4.575188
2	0.00000331	6.519828	0.000000620	0.000693	4.840733	0.000357	4.552668
3	0.00000359	6.555094	0.000000384	0.000812	4.909556	0.000317	4.501059
5	0.0000044	6.624282	0.000000572	0.000852	4.930440	0.000317	4.501059
7	0.00000534	6.727541	0.00000149	0.00113	3.053078	0.000317	4.501059
9	0.00000627	6.797268	0.00000045	0.00137	3.136721	0.000357	4.552668
11	0.00000935	6.970812	0.00000175	0.00127	3.103804	0.000396	4.597695
16	0.0000177	5.247973	0.00000197	0.00121	3.082785	0.000456	4.658965

Constants for Calcium:

$$x = 1.252$$

$$K = 0.07178$$

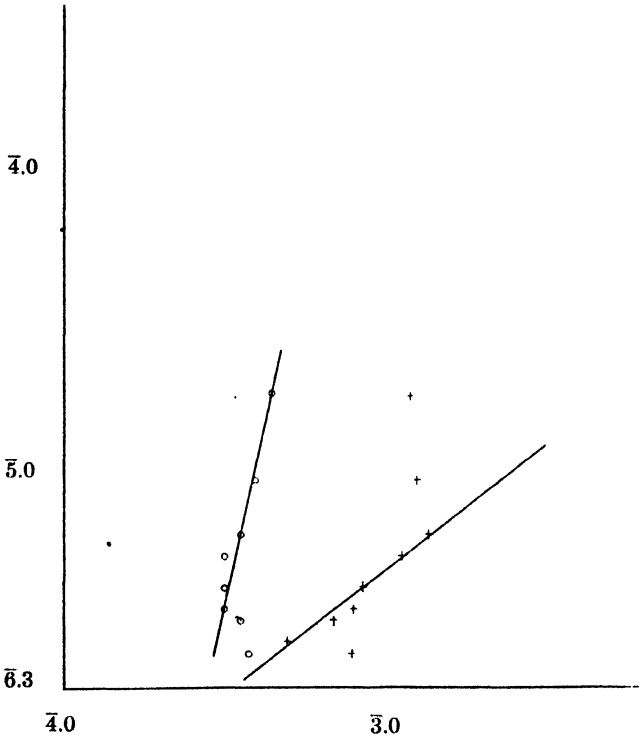


Figure 29.  
Granite and Azotobacter.  
(See Table 29)

TABLE 30

## CALCIUM SILICATE AND AZOTOBACTER

Time Days	C <sub>H</sub> , H ion Dextrose + Azotobacter	Log. H ion Dextrose + Azotobacter	H ion Calcium Silicate + Azotobacter	C <sub>Ca</sub> , Calcium Mols. per Liter	Log. Calcium Mols. per Liter
0	0.00000282	$\bar{6}.450249$	0.0000000778	0.00142	$\bar{3}.152288$
1	0.00000261	$\bar{6}.416641$	0.0000000778	0.00201	$\bar{3}.303196$
2	0.00000331	$\bar{6}.519828$	0.0000000912	0.00186	$\bar{3}.269513$
3	0.00000359	$\bar{6}.555094$	0.0000000912	0.00196	$\bar{3}.292256$
5	0.00000421	$\bar{6}.624282$	0.0000000112	0.00279	$\bar{3}.445604$
7	0.00000534	$\bar{6}.727541$	0.0000000941	. . . . .	. . . . .
9	0.00000935	$\bar{6}.797268$	0.000000107	0.00396	$\bar{3}.597695$
11	0.00000935	$\bar{6}.960812$	0.0000000778	0.00396	$\bar{3}.597695$
16	0.0000177	$\bar{5}.247973$	0.000000731	0.00406	$\bar{3}.695482$

Constants for Calcium:

$$x = 1,280$$

$$K = 0.03381$$



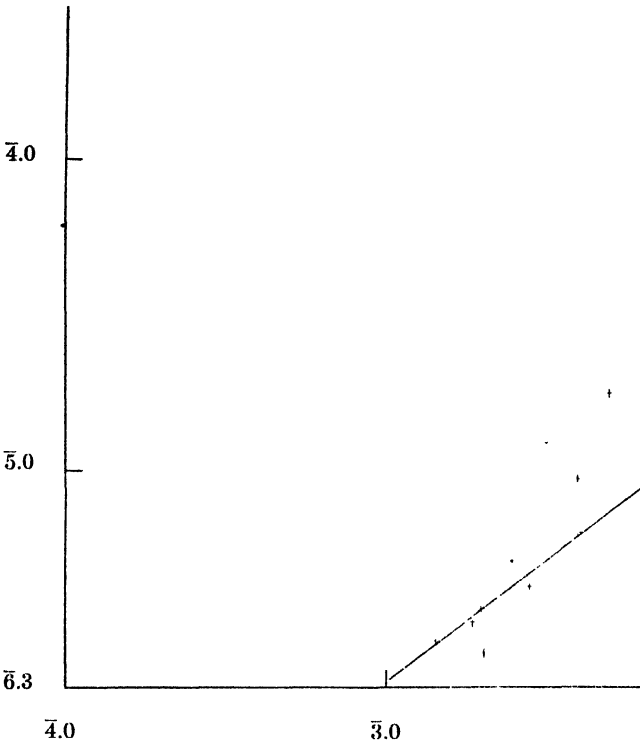


Figure 30.  
Calcium Silicate and Azotobacter.  
(See Table 30)

TABLE 31

## ORTHOCLASE AND B. COLI

Time Days	C <sub>b</sub> , H ion Dextrose +B. Coli	Log. H ion Dextrose +B. Coli	H ion Orthoclase +B. Coli	C <sub>a</sub> , Calcium Mols. per Liter	Log. Calcium Mols. per Liter
0	0.00000261	$\bar{6}.416641$	0.000000962	0.000694	$\bar{4}.841359$
1	0.000301	$\bar{4}.478566$	0.000141	0.000753	$\bar{4}.876795$
2	0.000486	$\bar{4}.686636$	0.000267	0.00206	$\bar{3}.313867$
3	0.000505	$\bar{4}.703291$	0.000339	0.00230	$\bar{3}.361728$
5	0.000505	$\bar{4}.703291$	0.000289	0.00224	$\bar{3}.350248$
7	0.000467	$\bar{4}.669317$	0.000278	0.00270	$\bar{3}.431364$
9	0.000414	$\bar{4}.617000$	0.000237	...	....
11	0.000467	$\bar{4}.669317$	0.000313	0.00282	$\bar{3}.450249$
16	0.000431	$\bar{4}.634477$	0.000179	0.00271	$\bar{3}.432969$

Constants for Calcium:

$$x = 1.690$$

$$K = 13,490.0$$

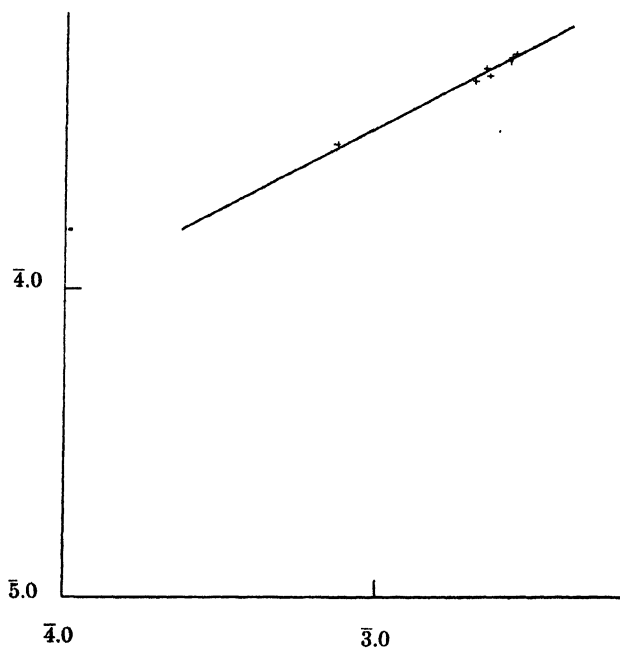


Figure 31.  
Orthoclase and B. Coli.  
(See Table 31)

TABLE 32  
BROITE AND B. COLI

Time Days	C <sub>b</sub> , H ion Dextrose +B. coli	Log. H ion Dextrose +B. coli	H ion Biotite +B. coli	C <sub>a</sub> , Calcium Mols. per Liter	Log. Calcium Mols. per Liter	C <sub>a</sub> , Mag- nesium Mols. per Liter	Log. Mag- nesium Mols. per Liter	C <sub>a</sub> , Potassium Mols. per Liter	Log. Potassium Mols. per Liter
0	0.00000261	6.416614	0.000000596	0.00176	3.245513	0.001096	3.039811	0.000525	4.720159
1	0.000301	4.478566	0.000210	0.00106	3.025306	0.000358	4.553883	0.000332	4.521138
2	0.000486	4.686636	0.000367	0.00186	3.269513	0.000807	4.906874	0.000424	4.627366
3	0.000505	4.703291	0.000431	0.00205	3.311754	0.000807	4.906874	0.000458	4.660865
5	0.000535	4.728354	0.000398	0.00204	3.309630	0.000987	4.994317	0.000475	4.676694
7	0.000567	4.753583	0.000348	0.00225	3.352183	0.001006	5.002598	0.000486	4.686636
9	0.000584	4.766413	0.000348	0.00238	3.376577	0.001096	5.039811	0.000500	4.698970
11	0.000597	4.775974	0.000301	0.00240	3.380211	0.00115	5.060698	0.00050	4.698970
16	0.000579	4.762679	0.000382	0.00228	3.357935	0.001113	5.046495	0.00052	4.716003

Constants for Calcium:

$$x = 1.225$$

$$K = 8.299$$

Constants for Magnesium:

$$x = 1.870$$

$$K = 401,800$$

Constants for Potassium:

$$x = 0.808$$

$$K = 0.05784$$

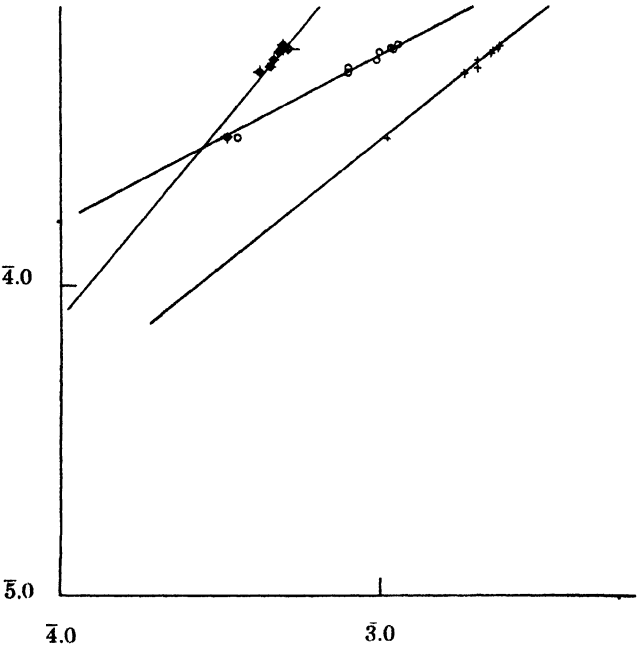


Figure 32.  
Biotite and B. Coli.  
(See Table 32)

TABLE 33

## GRANITE AND B. COLI

Time Days	C <sub>h</sub> , H ion Dextrose +B. coli	Log. H ion Dextrose +B. coli	H ion Granite +B. coli	C <sub>a</sub> , Calcium Mols. per Liter	Log. Calcium Mols. per Liter	C <sub>h</sub> , Iron Mols. per Liter	Log. Iron Mols. per Liter
0	0.0000261	̄6.416614	0.000000596	0.000654	̄4.815578	0.000792	̄4.898725
1	0.000301	4.478566	0.000153	0.000841	4.924796	0.00127	̄3.103804
2	0.000486	4.686636	0.000278	0.00120	̄3.079181	0.00161	̄3.26826
3	0.000505	̄4.703291	0.000376	0.00131	̄3.117271	0.00198	̄3.296665
5	0.000505	4.703291	0.000278	0.00137	̄3.136721	0.00335	̄3.525045
7	0.000467	4.669371	0.000289	0.00146	̄3.164353	0.00394	̄3.595496
9	0.000414	̄4.617000	0.000237	0.00150	̄3.116091	0.00410	̄3.612784
11	0.000467	4.669317	0.000339	0.00156	̄3.193125	0.00411	̄3.613842
16	0.000431	4.634477	0.000172	0.00153	̄3.184691	0.00408	̄3.610660

Constants for Calcium:

$$x = 0.875$$

$$K = 0.06502$$

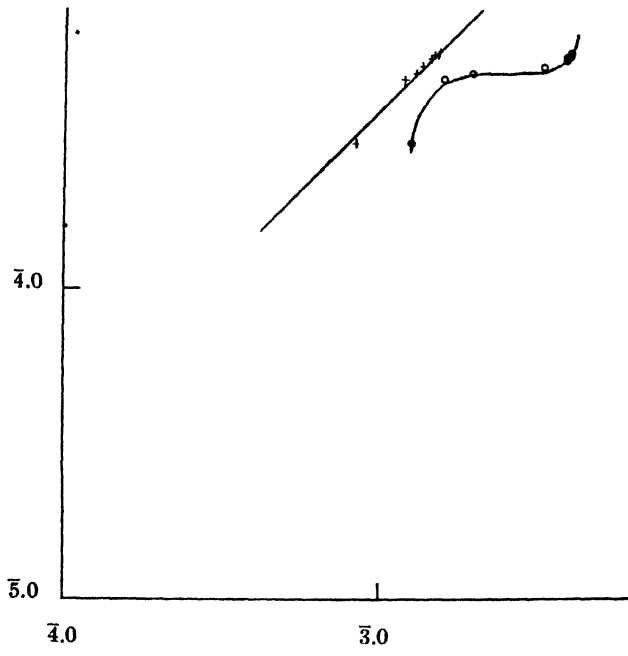


Figure 33.  
Granite and B. coli.  
(See Table 33)

TABLE 34  
CALCIUM SILICATE AND B. COLI

Time Days	Ch. H ion Dextrose +B. coli	Log. H ion Dextrose +B. coli	H ion Calcium Silicate +B. coli	Ca. Calcium Mols. per Liter	Log. Calcium Mols. per Liter
0	0.000000261	6.416614	0.000000220	0.00337	3.527630
1	0.000301	4.478566	0.0000716	0.0108	2.033424
2	0.000486	4.686636	0.0000564	0.0397	2.598791
3	0.000505	4.703291	0.0000687	0.0526	2.720986
5	0.000535	4.728354	0.0000806	0.0723	2.859138
7	0.000567	4.753583	0.0000745	0.0848	2.928396
9	0.000584	4.766413	0.0000716	0.0972	2.987666
11	0.000597	4.775974	0.0000716	0.1018	1.007748
16	0.000579	4.762679	0.0000635	0.1238	1.092721

Constants for Calcium:

$$x = 5.070$$

$$K = 1,667. \times 10^{11}$$



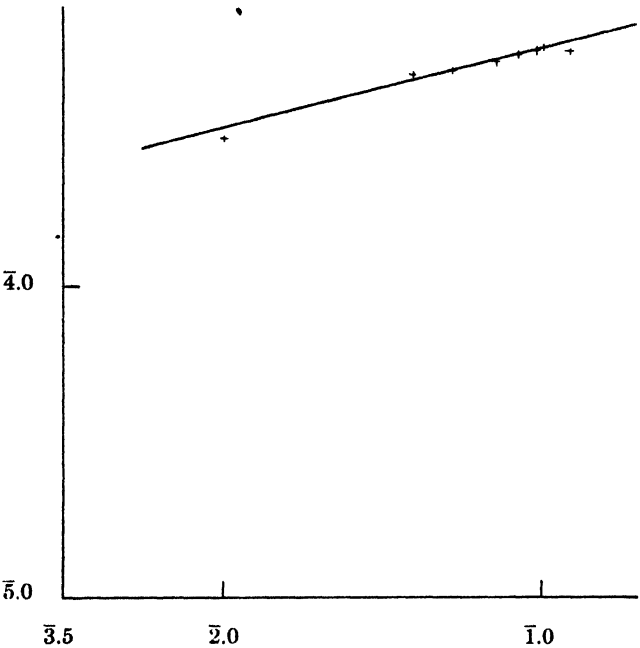


Figure 34.  
Calcium Silicate and B. coli.  
(See Table 34)

TABLE 35

## ORTHOCLASE AND B. LACTIS ACIDI

Time Days	C <sub>b</sub> , H ion Dextrose +B. lactis acidi	Log. H ion Dextrose +B. lactis acidi	H ion Orthoclase +B. lactis acidi	C <sub>a</sub> , Calcium Mols. per Liter	Log. Calcium Mols. per Liter
0	0.00000421	̄6.624282	0.000000354	0.00147	̄3.167317
1	0.0000157	̄5.195900	0.00000935	0.000912	̄4.959995
2	0.000228	̄4.357935	0.00000967	0.000852	̄4.930440
3	0.000398	̄4.599883	0.00000967	0.00162	̄3.209515
5	0.000642	̄4.807535	0.0000217	0.00228	̄3.357935
7	0.000696	̄4.842609	0.0000170	0.00235	̄3.371068
9	0.000696	̄4.842609	0.0000192	0.00249	̄3.396199
11	0.000789	̄4.897077	0.0000244	0.00271	̄3.432969
16	0.000642	̄4.807535	0.0000286	0.00210	̄3.322219

Constants for Calcium:

$$x = 0.867$$

$$K = 0.0006652$$

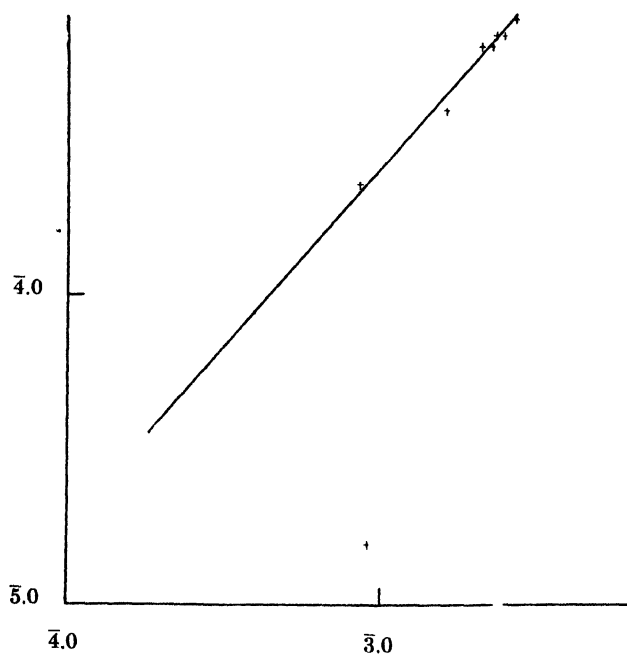


Figure 35.  
Orthoclase and B. lactis acidi.  
(See Table 35)

TABLE 36  
BROTITE AND B. LACTIS ACIDI

Time Days	Ca, H ion Dextrose+ B. lactis acidi	Log. H ion Dextrose+ B. lactis acidi	H ion Biotite+ B. lactis acidi	Ca, Calcium Mols. per Liter	Log. Calcium Mols. per Liter	Ca, Mag- nesium Mols. per Liter	Log. Mag- nesium Mols. per Liter	Ca, Potassium Mols. per Liter	Log. Potassium Mols. per Liter
0	0.00000421	6.624282	0.000000195	0.000872	4.940516	0.000144	4.158362	0.00040	4.602060
1	0.0000157	5.195900	0.000000787	0.00101	3.004321	0.000216	4.334454	0.00051	4.707570
2	0.000228	4.357935	0.00000149	0.000712	4.852480	0.000252	4.401401	0.00062	4.792392
3	0.000398	4.599883	0.000000727	0.00103	3.012837	0.000269	4.429752	0.00054	4.732314
5	0.000462	4.807535	0.00000241	0.00129	3.110590	0.000264	4.421604	0.00066	4.819544
7	0.000496	4.842609	0.00000306	0.00139	3.143015	0.000276	4.440909	0.00070	4.845098
9	0.000496	4.842609	0.00000345	0.00137	3.136721	0.000282	4.450249	0.00073	4.863323
11	0.000789	4.897077	0.00000653	0.00155	3.190332	0.000272	4.434569	0.00068	4.832509
16	0.000642	4.807535	0.00000967	0.00139	3.143015	0.000296	4.471292	0.00070	4.845098

Constants for Calcium:

$$x = 0.564$$

$$K = 0.00003190$$

Constants for Magnesium:

$$x = 0.096$$

$$K = 0.000001637$$

Constants for Potassium:

$$x = 0.106$$

$$K = 0.0000007569$$

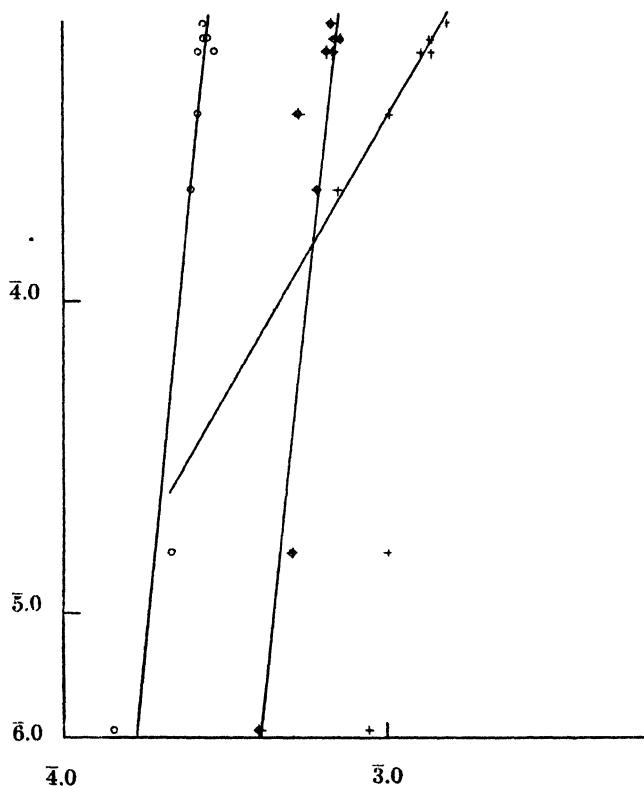


Figure 36.  
Biotite and *B. lactis acid*.  
(See Table 36)

TABLE 37

## GRANITE AND B. LACTIS ACIDI

Time Days	C <sub>a</sub> H ion Dextrose+ B. lactis acidi	Log H ion Dextrose+ B. lactis acidi	H ion Granite+ B. lactis acidi	C <sub>a</sub> Calcium Mols. per Liter	Log Calcium Mols. per Liter	C <sub>a</sub> Iron Mols. per Liter	Log Iron Mols. per Liter
0	0.0000421	5.624282	0.00000187	0.000317	4.501059	0.000218	4.338456
1	0.0000157	5.195900	0.00000627	0.000773	4.888179	0.000317	4.501059
2	0.000228	4.357985	0.00000735	0.000693	4.840733	0.000635	4.802774
3	0.000398	4.599883	0.00000653	0.000734	4.865696	0.000614	4.788168
5	0.000642	4.807535	0.0000192	0.000753	4.876795	0.000892	4.950365
7	0.000696	4.842609	0.0000177	0.000773	4.888179	0.00101	5.004321
9	0.000696	4.842609	0.0000192	0.000734	4.865696	0.000982	4.992111
11	0.000789	4.897077	0.0000192	0.000753	4.876795	0.00101	4.876795
16	0.000642	4.807535	0.0000192	0.000795	4.900367	0.00117	5.068186

Constants for Calcium:

$$x = 0.067$$

$$K = 0.000003846$$

Constants for Iron:

$$x = 0.291$$

$$K = 0.00001002$$

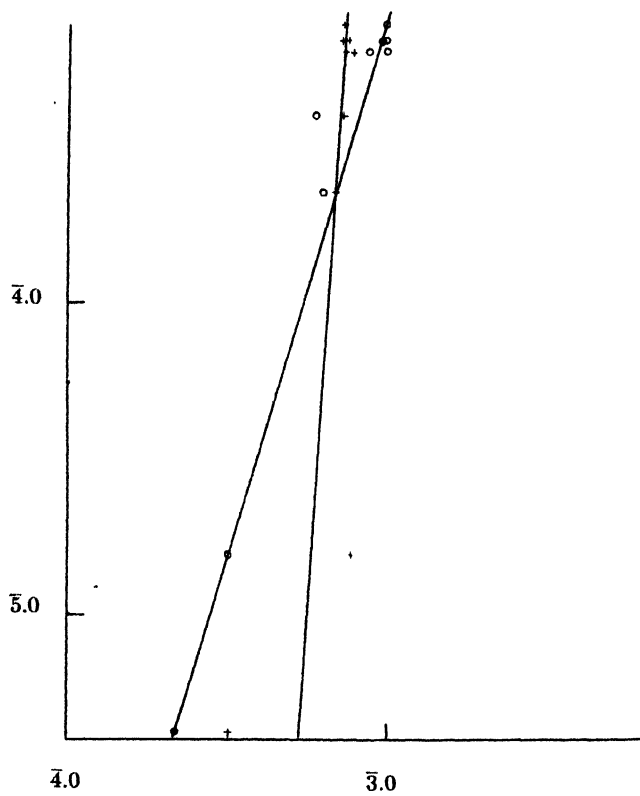


Figure 37.  
Granite and *B. lactis acidi*.  
(See Table 37)

TABLE 38  
CALCIUM SILICATE AND B. LACTIS ACIDI

Time Days	Ch. H ion Dextrose +B. lactis acidi	Log. H ion Dextrose +B. lactis acidi	H ion Calcium Silicate +B. lactis acidi	C <sub>2</sub> , Calcium Mols. per Liter	Log. Calcium Mols. per Liter
0	0.00000421	6.624282	0.0000000877	0.00229	3.359835
1	0.0000157	5.195900	0.000000136	0.00248	3.394452
2	0.000228	4.357935	0.000000142	0.00475	3.676694
3	0.000398	4.599883	0.0000000778	0.00416	3.619093
5	0.000642	4.807535	0.0000000778	0.00486	3.686636
7	0.000696	4.842609	0.0000000411	0.00554	3.743510
9	0.000696	4.842609	0.0000000544	0.00545	3.736397
11	0.000789	4.897077	0.0000000544	0.00525	3.720159
16	0.000642	4.807535	0.0000000544	0.00565	3.752048

Constants for Calcium:

$$x = 0.170$$

$$K = 0.0000002710$$



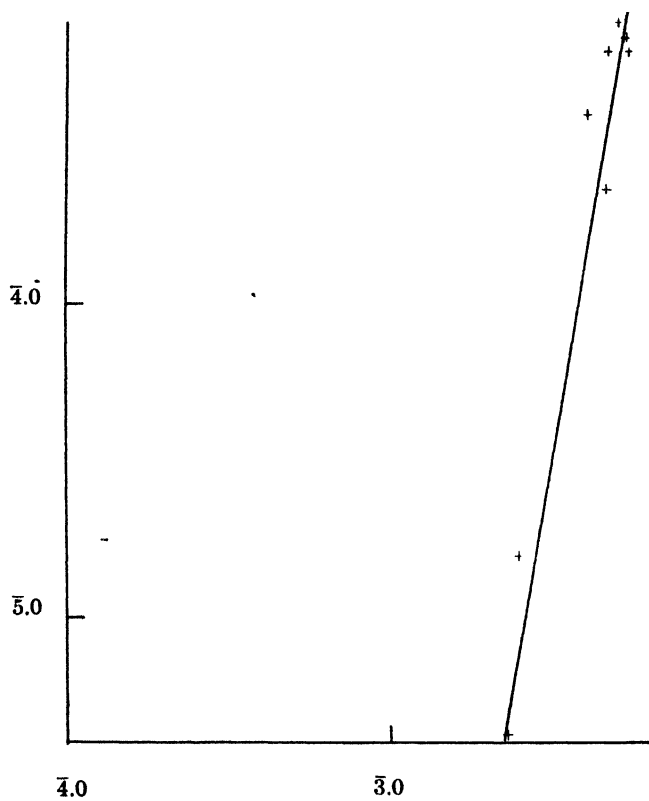


Figure 38.  
Calcium Silicate and *B. lactis acidi*.  
(See Table 38)



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June 30, 1922

STUDIES ON A DRAINED MARSH SOIL  
UNPRODUCTIVE FOR PEAS

BY  
PAUL S. BURGESS

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INTRODUCTION

The unparalleled progress made during recent years in chemistry and physics has given decided impetus to the development of scientific methods in soil science and in the science of plant physiology, which are rapidly supplanting the older, more empirical methods of experimentation.<sup>28\*</sup> New and improved procedures are constantly appearing for the elucidation of problems involving a lack of soil fertility, while the fundamental questions of plant nutrition are being investigated with thoroughness and the results interpreted with discriminating care.

\* Literature cited, pp. 393 to 396.

At present, the more important factors recognized as bringing about a state of infertility in soils are:

1. Untoward climatic conditions.
2. Too slight a concentration, at some time during the growth period, of one or more essential mineral elements dissolved in the soil solution, or the lack in the solution of a proper physiological balance of ions or salts.
3. The presence of substances dissolved in the soil solution which may be toxic to plant growth; these may be either of organic or inorganic nature.
4. Poor physical conditions obtaining in either the surface or the subsoil.
5. A condition of either abnormal or subnormal activity on the part of certain of the soil's micro-organic population.
6. The absence of sufficient quantities of organic materials undergoing active decomposition.

Until comparatively recently, agricultural chemists and students of plant nutrition have accepted the earlier and more obvious explanations of most of these facts without question, while the newer conceptions went unproved and unchallenged. Now, however, studies of cell permeability are being made, the questions of antagonism between ions, and of proper physiological balance between salts in both soils and solution cultures are being considered, while explanations of such observations are being advanced. The rapidity with which a soil is able to replenish or renew solutes absorbed from its solution, as well as the total concentration at any given time during the growth period, is now recognized as of extreme importance to continued crop production. The use of the conductivity apparatus and the cryoscopic method has given much valuable comparative data along these lines, and has opened fields heretofore unexplored, while delicate quantitative methods have also been perfected in this connection. Great advances have recently been made in the study of the nature of soil acidity as well as in methods for its accurate determination. And finally, the recent investigations in the realm of soil colloids—the effects upon the colloids of salt applications, as well as the direct effects of the colloids themselves in regulating the concentration of the soil's solution, and in modifying its moisture relations—should receive merited attention.

Armed with this knowledge, the soil scientist is today better able than ever to cope with the many obscure and puzzling problems of

low productivity in soils, which, although everywhere encountered, are especially apparent in the more arid or semi-arid sections of this country. The application of these modern methods to the solution of practical field problems now demands our attention if their benefits are to be of direct value to the practice of agriculture. To this end, the experiments herein described were undertaken.

### STATEMENT OF THE PROBLEM

Large areas of tidewater and overflow lands bordering the San Pablo and San Francisco bays and the Sacramento and San Joaquin rivers have in the past been drained and are at present used to grow a variety of crops. Certain areas within these reclaimed sections, varying in extent from an acre to many hundreds of acres, are unproductive for certain crops. The study discussed in this paper deals with a careful investigation of one partially unproductive area comprising about a thousand acres, located at Ignacio, California, on the property of the California Packing Corporation. The owners of this ranch were especially desirous of growing peas for canning purposes on the land under experiment, but have had very poor crops during the past few years. The peas ordinarily sprout and come up well, but when five or six inches high, turn yellow and gradually die. A few plants of each crop always mature, but hardly a third of a normal crop usually is harvested. When we consider that there are thousands of acres of similar lands in California which have been drained and brought under cultivation at great expense, the importance of a careful and thorough study of this problem can hardly be over-emphasized.

### METHODS EMPLOYED

As has been stated, one of the main objects of the present investigation was to test the applicability of certain modern methods of soil research to the solution of a practical field problem. Among those methods which have recently come into considerable prominence may be cited the periodical-water-extraction procedure, which has been largely developed and standardized by the work of Burd,<sup>5</sup> Hoagland,<sup>21</sup> and Stewart.<sup>44</sup> The water extraction idea for soil investigations is not a new one. It has been used in Europe for over sixty years,<sup>\*</sup> and

<sup>\*</sup> An extensive bibliography is given by Stewart.<sup>44</sup>

twenty years ago in this country, King<sup>25, 26</sup> applied it to comparative fertility work in the field. Also, in the method proposed by Burd and his associates the extraction procedure and certain other details are quite similar to those used by our Federal Bureau of Soils many years ago. The difference between the two lies in the manner of application to the problem, and in the method of interpreting the results. One of the chief points of weakness attaching to the procedures of the earlier workers, and never satisfactorily overcome by them, has now been surmounted through the careful and painstaking work of Stewart,<sup>44</sup> Hibbard,<sup>19, 20</sup> and others. I refer to methods of chemical analysis of the soil extract. In the earlier work, analytical methods were usually far too crude to differentiate between the slight differences often obtaining. Inaccurate colorimetric methods were then the rule. Today, these have largely been supplanted by volumetric and gravimetric procedures which insure more accurate results. The general method of experimentation mentioned above is given in detail by Stewart.<sup>44</sup>

During the past few years several field tests with fertilizers have been made upon the soil under study. The application of lime has occasionally increased yields somewhat, and the addition of superphosphate has consistently improved conditions, although the cost of the applications has not always been met. A preliminary examination made by the writer showed the soil to be very acid in reaction, while the deeper layers of the subsoil carried large quantities of the "white alkali" salts, notably sulfates.

With the results and methods just discussed in mind, it was decided to conduct two series of experiments: first, a set of plot tests in the field, applying superphosphate to certain plots and liming others to neutrality, proper checks being maintained; and, second, a pot experiment with various soil amendments, to be carried out in the greenhouse on the campus of the University of California; the same soil to be used and peas to be grown in both cases. The two crops were to be planted at the same time, and soil samples were to be drawn periodically from each, extracted and analyzed. Soil reaction under the growing crops was also to be closely followed, while alkali determinations were to be made from time to time in the field soil. The presence or absence of soluble organic soil toxins could also be noted by the application of an excess of  $\text{CaCO}_3$ , for Truog and Sykora<sup>45</sup> have shown such poisonous constituents to be rendered innocuous in soils by the complete neutralization of soil acidity as well as by the use of certain other soil amendments.

Both the field and the pot soils were sampled every four weeks during the growing period except as noted below. A sample was drawn from directly beneath the row of growing plants, from four places in each plot (see fig. 1), care being taken to obtain a representative sample down to a depth of 7 inches (surface soil). The twelve individual samples from the checks and a like number from the phosphate plots were then mixed very thoroughly and quartered down for the final composite samples. These were brought at once to the laboratory, passed through a 2 mm. sieve, and placed in tight Mason

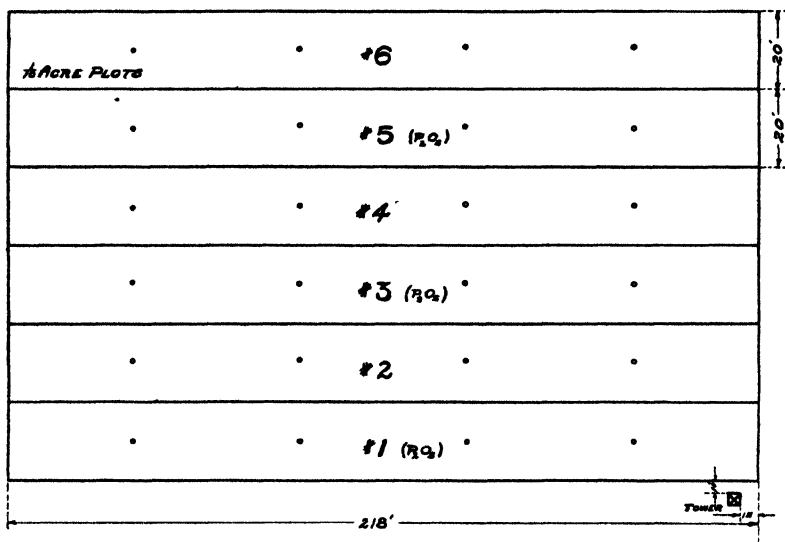


Fig. 1.—Method of sampling plot soils.

jars, after withdrawing sufficient soil for moisture determinations. The proper amounts of the moist soils, the percentages of moisture being taken into consideration, were then weighed out to make 300 g. of water-free soil, to which sufficient distilled water was added to bring the proportion of water to soil up to exactly 5 to 1. The mixtures of soil and water were now shaken for one hour in an end-over-end shaking machine, running at a speed of 7 revolutions per minute. Settling was allowed to take place overnight, after which the supernatant liquids were siphoned off and filtered through Pasteur-Chamberland filters. The resulting clear solutions were used for analysis by methods which will be given later. Hydrogen-ion determinations were made upon portions of the moist soils as soon as received at the laboratory. The hydrogen electrode described by Sharp and Hoagland<sup>42</sup> was employed.

In the greenhouse pot experiment a sharp small-bore 18-inch cheese trier was used in sampling, each core being taken from the entire depth of soil. In order to obtain sufficient soil for the water extractions, it was necessary to take three cores from each pot at each sampling. The resulting holes were always filled with similar dry, untreated soil. Proper precautions were employed to avoid subsequent sampling in the same places. The moist soils were placed at once in tight Mason jars and hydrogen-ion determinations and extracts were subsequently made in exactly the same way as described for the

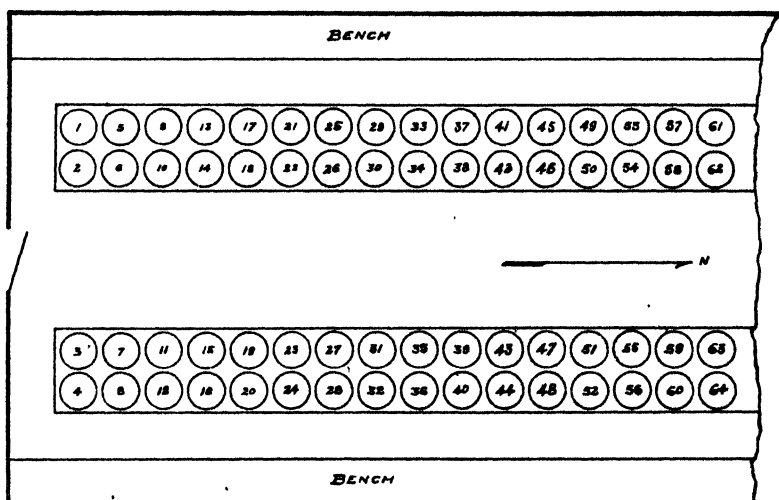


Fig. 2.—Arrangement of pots in greenhouse experiment.

field samples. Conductivity measurements were made upon the extracts at each sampling. The simple Kohlrausch conductivity outfit was employed. A detailed description of the apparatus, together with conversion tables, is given by Oswald and Luther<sup>39</sup> (pp. 461-477). The specific resistances (in ohms), rather than their reciprocals, the specific conductivities, have been employed in the work hereafter reported.

The clear soil extracts were regularly analyzed for the following ions, Ca, Mg, K,  $\text{PO}_4$  and  $\text{NO}_3$ , supplemented by occasional determinations of  $\text{SO}_4$ , Cl, and Al. Carbonates and bicarbonates were usually absent except where lime was used. The  $\text{PO}_4$ -, Ca-, and K-ions were determined in accordance with the methods proposed by Stewart<sup>44</sup> (pp. 328, 329), except that 600 cc. aliquots were found necessary in the case of  $\text{PO}_4$ -ion, while 400 cc. portions were used for Ca and K.



Nitrates and chlorides were run by the phenoldisulphonic acid method and by titration with a standard silver nitrate solution, respectively. These methods are essentially those widely used in sanitary water analysis.<sup>1</sup> Magnesium was determined gravimetrically as the pyrophosphate in the filtrate from the calcium oxalate precipitate, after first evaporating to dryness and burning off ammonium salts. Sulfates were determined gravimetrically, weighing as  $\text{BaSO}_4$ . Six hundred cc. aliquots of the original soil extracts were first evaporated to dryness, burned off, and taken up in very dilute hydrochloric acid before precipitation with barium chloride. The water-soluble sodium, silica, and aluminum were occasionally determined. In all cases aliquots were taken large enough to make possible the use of standard gravimetric procedures.

At the beginning of the work many determinations were made upon identical extracts in order to check up the results as regards accuracy of duplication. As a rule, the larger the amounts of the various ions present, the more accurate would be the determinations. For instance, in the case of K, when that ion was present above 40 p. p. m. (as was usually the case), duplicate determinations invariably checked within less than 3 p. p. m. In other words, the percentage variation between duplicates was here under 8 per cent. Better checks than this were usually obtained with Ca, Mg,  $\text{NO}_3$ , and Cl, while with  $\text{PO}_4$ , duplicates might differ by 1 p. p. m. when present in quantities of less than 5 p. p. m. The very small amounts of phosphorus always present in these soil extracts made this element unusually difficult to determine accurately. Sulfates always checked well in duplicate determinations.

Stewart<sup>44</sup> (pp. 332, 333) has discussed very fully the form, or method, of recording final results, and until more is definitely known about the true soil solution, it seems best to the writer, also, to express all results as "parts per million of dry soil." This procedure has been followed throughout.

## THE FIELD EXPERIMENTS

### *The Soil Type*

The soil at Ignacio has been formed by the deposition of clay and very fine silt brought down by the Sacramento River. It is a light drab clay loam underlain at a depth of six to seven inches by a very deep, almost impervious, clay subsoil of lighter color. Neither the

surface nor the subsoil contains gritty particles of any kind and when wetted, both are extremely smooth, plastic, and sticky. Aeration is thus always poor and deep root development impossible. The apparent specific gravity of the surface soil when air dried and heavily compacted is 0.970. An acre to a depth of 6 inches thus weighs about 1,320,000 pounds or 660 tons. Its light weight is due chiefly to the 13 per cent of organic matter which it contains. The total water holding capacity (Hilgard method) is 104% of the moisture-free soil. The optimum moisture holding capacity is thus not far from 50% while the hygroscopic coefficient is 14%.

Chemically, the soil presents a number of very interesting features. An analysis made by the Hilgard method (digestion for 40 hours at 100° C. in HCl, sp. gr. 1.115) on a representative composite sample from the poor area under study appears in Table I. Only those elements important to a discussion of plant nutrition were determined. The table also gives the amounts, in parts per million, of the various ions (computed as the oxides for comparison) soluble in water, determined by the methods previously described.

TABLE I  
CHEMICAL ANALYSIS OF IGNACIO SOIL  
(Reduced to water-free basis)

	Strong Acid Soluble Per Cent	Water- Soluble p. p. m.
Insoluble matter (SiO <sub>2</sub> ) .....	58.60	55
Potash (K <sub>2</sub> O) .....	0.23	54
Soda (Na <sub>2</sub> O) .....	.....	286
Lime (CaO) .....	0.66	125
Magnesia (MgO) .....	1.34	75
Iron (Fe <sub>2</sub> O <sub>3</sub> ) .....	21.15	none
Alumina (Al <sub>2</sub> O <sub>3</sub> ) .....		24
Phosphoric Acid (P <sub>2</sub> O <sub>5</sub> ) .....		4
Sulfuric Acid (SO <sub>3</sub> ) .....	0.62	400
Total nitrogen (N) .....	0.36	.....
Nitrates (NO <sub>3</sub> ) .....	.....	150
Chlorine (Cl) .....	.....	100
Loss on ignition (volatile) .....	13.25	.....

Manganese was practically absent, as were also carbonates and bicarbonates. Negative tests were noted for ferrous salts.

These results are inserted merely to show the general chemical composition and the relative solubilities of this soil. It is interesting to observe the reversed "lime-magnesia ratio" when total percentages of these compounds are compared with their water-soluble portions;

also that the phosphoric acid, although not low in total percentage, is but very slightly soluble in water. High amounts of available potassium as well as the presence of considerable quantities of water-soluble aluminum are also shown.

As this soil was at one time below the level of San Pablo Bay, it was thought desirable at the inception of the work to make a careful alkali survey of the area, especially of that portion of it where it was later planned to conduct the field experiment. Accordingly, about 40 samples of surface soil were taken. Several borings were also made to a depth of 5 feet, the 1-foot samples being segregated and quantitatively analyzed for water-soluble chlorides and sulfates. Carbonates were absent. Bicarbonates were present in traces only. Table II presents the data secured. The figures for the surface soil are averages of 40 analyses, all of which agreed fairly closely. The subsoil samples (except top foot) are averages of duplicate borings. The number of samples averaged appears in the table.

TABLE II  
ALKALI DETERMINATIONS

	NaCl Per Cent.*	Na <sub>2</sub> SO <sub>4</sub> Per Cent.*
Surface, 6 to 7 inches (40 samples) .....	0.018	0.066
Sub-soil, 1st foot (20 samples) .....	.100	.180
Sub-soil, 2d foot (2 samples) .....	.150	Heavy test†
Sub-soil, 3d foot (2 samples) .....	.450	Heavy test
Sub-soil, 4th foot (2 samples) .....	.710	Heavy test
Sub-soil, 5th foot (2 samples) .....	.880	Heavy test

The percentages of alkali present in the surface soil, although considered small, may possibly approach toxic concentrations where limited moisture conditions prevail. The large quantities of soluble salts in the subsoil probably exert no direct effect, for plants are seldom able to root there below 12 inches on account of the impervious, compact condition of the soil. That alkali and subsequent leaching have in the past contributed to these untoward conditions is probable. The work of Sharp,<sup>41</sup> as well as that of Hager,<sup>15</sup> has shown that soils, especially heavy clays, once saturated with solutions of soluble salts, or inundated with sea water and later washed free, are almost invariably left in a very poor and impervious physical condition.

\* Percentages figured to *dry soil basis*. The surface soil contained 6% water (air dry), while the subsoil carried an average of 34% water when received at the laboratory.

† The writer did not quantitatively determine the sulfates in these samples.

The next factor to receive attention was soil reaction. Peas were to be grown and, in the past, a neutral or slightly alkaline reaction has been advocated for this crop. The Ignacio soil was found to be extremely acid. The hydrogen-ion concentration, as determined on a large number of fresh field samples, gave an average exponential value of  $P_H = 4.46$ . A preliminary experiment to ascertain the approximate lime requirement was performed after the method of electrometric titration with a standard solution of  $\text{Ca}(\text{OH})_2$  as proposed by Sharp and Hoagland.<sup>42</sup> Considering an acre-six-inches of this soil to weigh 660 tons (see above), it was found that 4 tons of calcium carbonate were immediately required to neutralize the concentration of hydrogen-ion present.\* An experiment was now set up using 100-gram portions of the field soil and thoroughly mixing each with different amounts of pure  $\text{CaCO}_3$ . Optimum moisture conditions were maintained. Table III shows the lime treatments, together with the  $P_H$  values as determined from time to time.

TABLE III  
LIME REQUIREMENT OF FIELD SOIL

Number	Tons $\text{CaCO}_3$ per-Acre	Grams $\text{CaCO}_3$ per 100 Grams Soil	$P_H$			
			After 1 week	After 1 month	After 5 months	After 7 months
1	3	0.45	6.3	5.7	5.5	5.4
2	4	0.60	7.0	6.0	5.9	5.9
3	5	0.75	7.1	5.3	6.1	5.5
4	6	0.90	7.2	6.6	6.6	6.5
5	8	1.20	7.4	7.1	7.2	7.1
6	10	1.50	7.6	7.2	7.2	7.2

Considering  $P_H = 7$  to indicate a state of neutrality, a glance at this table shows that a field application of 7 or 8 tons of lime carbonate per acre would be necessary to insure a slightly alkaline soil reaction for approximately the growing period of a crop. The fact that larger and larger amounts of lime are required upon standing would indicate that hydrogen-ions are being progressively and rapidly formed. This may be due to a decomposition of organic matter with subsequent formation of nitric acid and the less soluble organic acids, to silicate degradation, or to the hydrolysis of soluble aluminum compounds.

\* Recent work<sup>4, 24</sup> has intimated that there may be a relation between  $P_H$  and lime requirement, whereby the latter may be indirectly and rapidly determined, but it appears to the writer, as well as to Knight,<sup>27</sup> that much work still remains to be done before any general comparisons are possible.

### *Variability of the Field Soil*

It is in no wise the intention of the writer to enter into a detailed discussion of the factor of variability as applied to the study of field soils. The work herein reported was planned for other purposes. Variability studies have been recently attempted by Waynick<sup>47</sup> and by Waynick and Sharp<sup>48</sup> with some measure of success. That seemingly uniform soils may vary greatly both chemically and biologically within very small areas has been well and forcibly brought out by these investigators, and, as an excellent opportunity was here presented for obtaining further data along this line (where water extracts were concerned), a number of analyses are reported showing, for the field soil under discussion, the varying tendencies of the "total soluble solids," the Ca-, K-, NO<sub>3</sub>-, and the Cl-ions. The location chosen for the field plot experiment was the area whence these samples came and was in all respects as uniform in texture, color, and appearance as one could well find. It was unusually level, being comparatively free from slight local elevations or depressions so often present in otherwise uniform fields. For miles in all directions but slight visible differences could be detected. The locations of samples are shown in the accompanying diagram of the field plots.

DIAGRAM OF FIELD PLOTS

1-10th acre plots					1-5th acre plots				
x	x	6	x	x	x	x	x	x	x
x	x	5	x	x	x	9	x	x	x
x	x	4	x	x	x	x	x	x	x
x	x	3	x	x	x	8	x	x	x
x	x	2	x	x	x	x	x	x	x
x	x	1	x	x	x	7	x	x	x

Table IV presents the analytical results secured. Before computing the results as presented, they were in each case plotted and shown to form a proper frequency curve. This justifies the use of the statistical method in connection with these data.

As will be seen, not all of the 48 samples were analyzed in each case, but sufficient determinations were made to show prevailing con-

ditions. As duplicate extractions of the same sample seldom varied by more than 8% to 10% for any of the ions determined (and often by much less), and as the coefficient of variability (which is nothing more than the standard deviation expressed as its percentage of the mean or arithmetical average) varies from 12% in the case of K-ion to over 44% in the case of  $\text{NO}_3$ -ion, there can be no doubt that apparently uniform field soils are likely to vary greatly from place to place in water-soluble constituents; and it is evident that only averages of very large numbers of single determinations or analyses of carefully composited samples drawn from a considerable number of separate, uniformly distributed stations over areas under examination can give dependable results or significant differences. Thus the work of

TABLE IV  
VARIABILITY OF THE FIELD SOILS AS REGARDS MINERALS  
(Parts per million of dry soil)

Calcium-ion (Ca)	Potassium-ion (K)	Total Sol. Solids	Nitrate-ion ( $\text{NO}_3$ )	Chloride-ion (Cl)	Sulfate-ion ( $\text{SO}_4$ )
62	57	3215	310	120	986
49	54	2540	265	111	520
51	44	2350	288	110	296
48	61	2125	350	120	444
340*	44	3175	243	125	574
55	44	2300	177	130	499
59	54	2300	199	120	355
59	44	1975	133	130	383
278*	53	2500	221	125	316
92	56	2000	203	125	358
440*	48	1950	350	111	432
55	44	1475	203	125	327
60	48	3075	221	310*	381
55	55	2175	420	110	361
66	42	1825	310	105	449
52	39	2675	265	95	358
62	44	1800	221	95	419
59	46	2700	203	100	386
51	49	1975	203	95	363
50	40	1525	111	93	367
70	49	1250	88	90	399
63	48	3000	99	85	385
481*	42	1825	88	75	348
50	39	—	111	80	366
201*	56	Mean = $2244 \pm 74$	221	100	460
69	56	S.D. = $527 \pm 52$	133	90	424
60	55	C.V. = $23.5 \pm 2.3\%$	155	95	659
56	53	P.E. = $\pm 355$	111	100	378
87	—	—	97	90	388
75	Mean = $49 \pm 67$	—	97	85	658
69	S.D. = $6 \pm 53$	—	177	86	399
191*	C.V. = $12.2 \pm 1.1\%$	—	115	90	644
—	P.E. = $\pm 4.0$	—	177	100	392
Mean = $61 \pm 1.5$	—	—	142	150	409
S.D. = $10.9 \pm 1.1$	—	—	97	95	611
C.V. = $18 \pm 1.7\%$	—	—	115	75	457
P.E. = $\pm 7.4$	—	—	350	85	—
—	—	—	155	100	Mean = $428 \pm 10.9$
—	—	—	155	100	S.D. = $95 \pm 7.7$
—	—	—	111	75	C.V. = $22.3 \pm 1.8\%$
—	—	—	—	—	P.E. = $\pm 64.4$
—	—	—	Mean = $192 \pm 9.2$	Mean = $102 \pm 1.9$	—
—	—	—	S.D. = $86 \pm 6.4$	S.D. = $17.6 \pm 1.3$	—
—	—	—	C.V. = $44.8 \pm 3.4\%$	C.V. = $17.3 \pm 1.3\%$	—
—	—	—	P.E. = $\pm 58.0$	P.E. = $\pm 11.8$	—

\*Omitted from mean.

Waynick and Sharp on soil variability\* has been confirmed and shown to hold for certain water extractable materials as well as for nitrates, total nitrogen and organic carbon.

### *Results of the Plot Experiments*

A brief history of the management of the area under study follows:  
1913 and 1914: Reclaimed from salt marsh by diking and drainage.

1915: Planted to grain hay. Good yields (3 to 3½ tons per acre).

1916: Planted to peas. Failure.

1917: About one ton per acre of "beet-lime" (85%  $\text{CaCO}_3$ ) added and peas again planted. Failure.

1918: Planted again to peas. At first the crop came along nicely, but about the middle of March, when the peas were two-thirds grown, they suddenly began to die out. Small application of lime apparently had little effect. The crop was a failure. After the peas failed the land was immediately plowed and beans were planted. A very poor crop resulted—about 700 pounds per acre.

1919: Sugar beets were grown. A poor crop resulted (between 3 and 4 tons of small beets per acre).

1920: A large part of the poor land was again planted to sugar beets.

Much care was exercised in locating the experimental plots and in their subsequent oversight and treatment. Neither fertilizers nor soil amendments had previously been applied to this area, although small applications (1 ton per acre) of lime had been used on adjacent sections. About one and one-fifth acres (a piece 125 x 450 feet) was measured off during the month of July, 1919, and after the removal of a poor crop of sugar beets, was plowed and disked prior to planting. Six one-tenth acre plots, 20 x 218 feet,† were laid out as were also three one-fifth acre plots, 40 x 218 feet (see diagram above). The smaller plots were numbered from one to six, and superphosphate (18.1% water-soluble and 20.0% total  $\text{P}_2\text{O}_5$ ) at the rate of 1 ton per

\* For a full and detailed account of the statistical method as applied to the interpretation of biochemical results the reader is referred to the papers of Waynick already cited, to Wood,<sup>50, 51</sup> and to Davenport.<sup>10</sup>

† The plots were made long and narrow to facilitate working and harvesting by standard machinery. Lyon<sup>50</sup> has also shown that long and narrow plots give most dependable results.

acre was applied to the odd plots while the even ones received no treatment (checks). The center one-fifth acre plot (Number 8) was left untreated while Number 7 received finely ground limestone (99.6%  $\text{CaCO}_3$ ) and Number 9, sugar beet lime (87%  $\text{CaCO}_3$ ), both at the rate of 10 tons per acre. These applications were slightly in excess of the lime requirements for the surface soil (see page 348). The phosphate and lime applications were thoroughly disked into the surface soil about two weeks before planting.

The peas (Horseford's Market Garden Variety) were planted on October 26, 1919, in rows 30 inches apart, one inch apart in the rows. There were thus 8 rows in the smaller and 16 rows in the larger plots.

Since an important part of the plot experiment was the observation of the varying concentration of the soil solution under both the fertilized and the untreated peas (as manifested by periodical analyses of soil extracts prepared from carefully taken representative soil samples), samples, taken as previously described, were drawn and analyzed on September 3, after the plots had been prepared but before the superphosphate had been applied, and subsequently as the data in Table V show. (See also graphs in figs. 3 and 4.)

TABLE V  
PERIODIC LABORATORY DATA ON FIELD PLOT SOILS

Dates of Sampling Soils	Conductivity Measurements. Specific Resistance in Ohms		Determinations of Plant Food Ions (p. p. m. dry soil)									
			Ca-Ion		Mg-Ion		K-Ion		NO <sub>3</sub> -Ion		PO <sub>4</sub> -Ion	
	1	2	1	2	1	2	1	2	1	2	1	2
9-3-19	3000	3000	61	61	45	45	49	49	150	150	5.2	5.2
11-3-19	2560	1382	75	153	46	136	45	99	133	177	5.2	19.5
1-20-20	2430	1497	54	134	42	109	51	83	155	177	4.6	8.7
2-21-20	2970	1855	50	160	34	106	47	83	133	133	1.5	4.5
3-27-20	2495	1792	56	167	52	113	54	83	49	35	2.3	3.7
4-26-20	3965	2162	26	96	20	74	32	58	0	5	2.0	3.0
5-24-20	3258	1895	46	150	37	93	36	54	10	5	2.3	2.8

In all cases No. 1 = check plots and No. 2 = phosphate-treated plots.

As the rainfall during the year 1919-1920 was below the normal average for Marin Meadows Ranch,\* and as the growth of the peas

\* The annual rainfall data for the past seven years, September 1 to September 1, follows:

1913-1914 .....	35.79 inches
1914-1915 .....	32.99 inches
1915-1916 .....	27.31 inches
1916-1917 .....	14.19 inches
1917-1918 .....	9.20 inches
1918-1919 .....	17.99 inches
1919-1920 .....	11.39 inches



was so largely dependent upon this factor, it was not thought always desirable to draw soil samples at exactly four-week intervals. The following brief summary shows the sampling dates and correlates with these the condition of the peas at those times.

September 3, 1919: First samples drawn. Plots staked out but no fertilizers yet applied.

November 3, 1919: First sampling since planting. As less than 0.3 of an inch of rain had fallen since planting, but few of the seeds had sprouted.

January 20, 1920: The peas were 2 to 3 inches high and a good stand had been secured. Over 4 inches of rain had fallen since last sampling, but the nights were cold (often below freezing), and the days were usually cloudy and cold.

February 21, 1920: Less than one inch of rain had fallen since January 20. The soil was very dry (moisture determinations showed but 27% in the surface soil and 42% in the subsoil). The plants had grown but an inch or two during the month and were often more or less wilted during the middle of the day. The nights were cold. Poor conditions for growth. There was no difference between the checks and the phosphate-treated plots.

March 27, 1920: The plants were looking well. About 3 inches of rain had fallen since last sampling. The vines on the check plots were 6 to 8 inches high while those on the phosphate-treated plots were 10 to 12 inches high. The lime-treated plots showed no improvement over the checks.

April 26, 1920: The plants were looking fairly well, although little rain had been recorded during the month past. The vines were covered with blossoms and filling pods. There was a noticeable difference in favor of phosphate-treated plots although the lime-treated plots showed no gain.

May 24, 1920: Peas about ready to cut. Vines turning yellow; pods well filled and dry. The soil had dried out and was very parched and hard. This was the last date of sampling.

The plots were harvested June 3. The yields obtained are shown in Table VI.

TABLE VI  
PLOT YIELDS

	Gross Weights, Dry Peas and Vines		Net Weights, Dry Peas	
	lbs.	lbs. per acre	lbs.	lbs. per acre
Average yield per $\frac{1}{10}$ acre plot (checks)....	627	6,270	153	1,530
Average yield per $\frac{1}{10}$ acre plot (superphosphate) .....	820	8,200	200	2,000
Average yield per $\frac{1}{5}$ acre plot (checks)....	1,010	5,050	340	1,700
Average yield per $\frac{1}{5}$ acre plot (sugar beet lime) .....	1,090	5,450	340	1,700
Average yield per $\frac{1}{5}$ acre plot (ground limestone) .....	1,090	5,450	330	1,650

It will be seen that liming to neutrality had no effect upon yields. This is in accordance with former field observations on this soil. A more extended discussion of the effects of the application of lime

will be given later in connection with results secured in the greenhouse where moisture conditions were optimum and where a more careful chemical control was possible. The superphosphate treatment increased the yield of peas by approximately 25 per cent. This increase about paid for the treatment, and a future residual effect may be expected. Possibly a larger amount of superphosphate would have given higher yields, for much was lost due to reversion (see below).

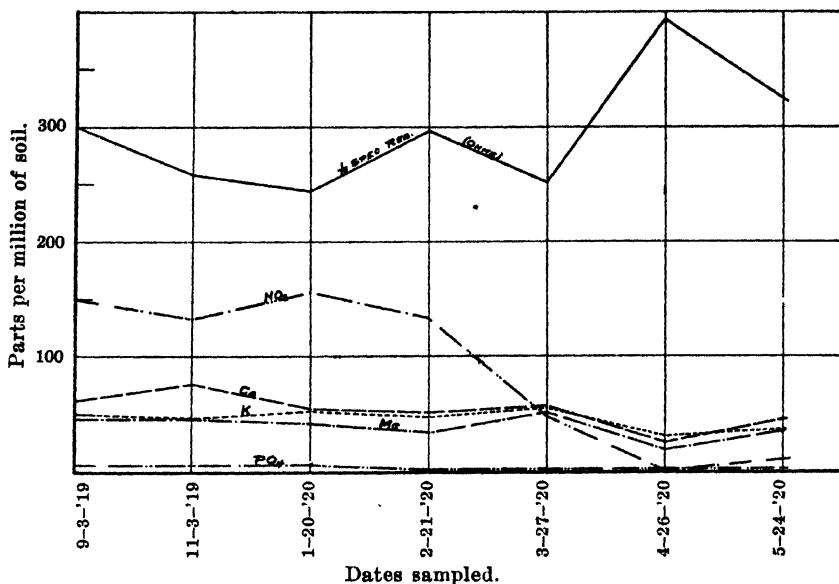


Fig. 3.—Water-soluble materials dissolved from unfertilized plot soils carrying pea crop.

As has been mentioned, the rainfall was subnormal throughout the entire growing period. That the low yields secured on both treated and untreated plots were attributable in large part to a lack of water will be shown by the following test. Four approximately 50-foot rows (two in a phosphate plot and two in a check plot) were chosen at random and regularly irrigated\* for a period of several weeks during the months of February and March. Rapid growth and great improvement over those plants not so treated was observed. As heavy rains fell during the latter part of March, irrigating was discontinued. The beneficial results of these few applications of water during the early stages of growth were, however, noticeable up to the time of harvesting.

\* Water hauled in a tank wagon.

The curves presented in figures 3 and 4 show graphically the rise and fall in concentration of the soil solution under the growing crop. In studying these graphs it should be remembered that the superphosphate was applied between September 3 and November 3 (see figure 4), and that the plants were absorbing nutrients most vigorously during the months of March and April. We note first that much greater concentrations of salts prevail throughout the entire period within the soils of the fertilized plots. This is clearly depicted by the solid line

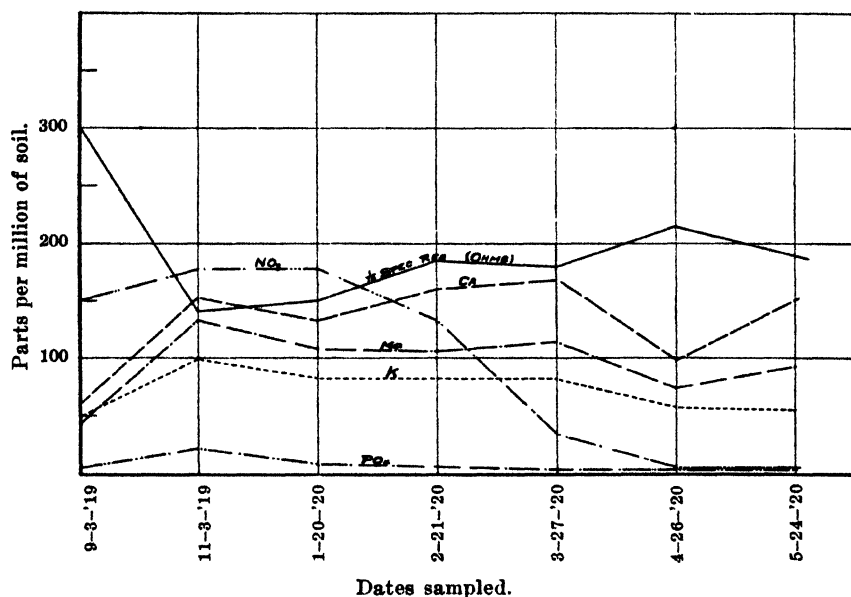


Fig. 4.—Water-soluble materials dissolved from phosphate-treated plot soils carrying pea crop.

representing one-tenth of the specific resistance in ohms. That this increased concentration is due in large part not to the superphosphate, but to the increased solubility of other ions caused by it within the soil itself, is strikingly shown by the Mg and K graphs. This doubtless accounts largely for the greater yields obtained on the phosphate plots, for where water is limited, Morgan<sup>37</sup> has shown that transpiration is necessarily less, and that the enhancing effect of fertilizers is relatively greatly increased. He states, "All fertilizers, both mineral and nitrogenous, have greatly decreased in their relative efficiency following an increase in soil moisture. The decrease is consistent." It is further a well established physiological fact that water is greatly economized by increasing the plant's supply of mineral salts (see Russel,<sup>40</sup> pages 29, 30).

Taking up the ions separately, we note that phosphate applications have but slightly affected nitrate formation. This is doubtless due to excessive soil acidity which dominates nitrification within this soil.

The yields show that soluble nitrogen is here more than adequate for peas. The amounts of potassium and magnesium, soluble in water, in this heavy clay soil, have been almost doubled throughout the entire growing period by the initial acid-phosphate application. That the gypsum present in the superphosphate is largely responsible for this increase is shown by results secured in the more carefully controlled greenhouse experiment (see McCool and Millar<sup>28</sup> in this connection). As would be expected, both water-soluble calcium and phosphorus have been somewhat increased in the soils of those plots receiving the soluble phosphate treatments.

Chlorides and sulfates were periodically determined. As these ions were always present in great excess, however, they have not been included in Table V nor in the graphs, but have been more properly figured as the sodium salts (white alkali), and appear in Table VII. An idea has prevailed in the past that occasional increases in the amount of alkali present may have been responsible for crop failures.

TABLE VII  
PERIODICAL DETERMINATION OF WHITE ALKALI IN PLOT SOILS

Date	% NaCl	% Na <sub>2</sub> SO <sub>4</sub>
September 3, 1919 .....	0.018	0.066
November 3, 1919 .....	.025	.....
January 20, 1920 .....	.023	.066
February 21, 1920 .....	.017	.067
March 27, 1920 .....	.032	.060
April 26, 1920 .....	.017	.050
May 24, 1920 .....	.020	.100

While the percentages of alkali here noted are doubtless innocuous if optimum moisture conditions exist, it is conceivable, as before stated, that at times of unusual drought, plants may suffer in the more concentrated soil solution that results, and, while a lack of water is directly responsible for this condition, alkali salts may well be considered an important indirect or contributing factor.

Other toxic compounds, as ferrous iron\* or soluble aluminum salts, here appealed to the writer as being possible causes of infertility. We were, however, unable to secure a positive test for ferrous iron in the surface soil. Special samples were taken for these tests, every

\* Certain heavy soils of the Transvaal have been shown by Watt<sup>46</sup> to have been rendered unproductive by accumulations of ferrous salts.

precaution being used to avoid oxidation in transit. On the other hand, water-soluble aluminum was usually found. Large numbers of determinations showed it to be present to the extent of 12 to 15 parts per million in the surface soil, while approximately twice these amounts were found in the subsoil.

The considerable literature upon the subject of aluminum toxicity has been notably extended during the past two or three years by the careful work of Hartwell and Pember,<sup>17, 18</sup> Conner,<sup>9</sup> Ames and Schollenberger,<sup>2</sup> and Miyake.<sup>35</sup> The first-mentioned investigators have definitely shown that soluble aluminum compounds exist in toxic concentrations in certain acid soils; that plants differ in their powers of resistance to soluble aluminum; and that such conditions may be readily ameliorated by applications of any substance which will precipitate aluminum-ion. From data furnished by Hartwell and Pember<sup>18</sup> (page 266), it is possible to calculate the concentration of soluble aluminum present in the acid soils upon which they experimented. This was found to be approximately 77 parts of  $Al_2O_3$  or 41 parts of soluble aluminum per one million parts of dry soil. They extracted using slightly different proportions (about 1 to 3) of soil and water than did the writer, but the results should be fairly comparable. They furthermore found that at least 15 p. p. m. of aluminum in solution cultures with growing plants were required to produce signs of toxicity. In the light of these results, it would appear somewhat doubtful whether the relatively small quantities (12 to 15 p. p. m. of aluminum) found in the soil of the Marin Meadows Ranch could be entirely responsible for the seriously depleted yields. The other authors cited in this connection have shown that amounts of aluminum greatly in excess of 15 p. p. m. of soil are necessary to render conditions toxic for crop plants in soils; and, finally, the plants in our own untreated pots, in which this soil was used without drainage, gave no indications of aluminum-poisoning.

To sum up briefly the points brought out by the field plot experiment, we may conclude with reasonable certainty that, during the past season at least, water has been the limiting factor in crop production; that a one-ton application of superphosphate in absence of irrigation has increased the yield of peas by approximately 25 per cent; that liming to neutrality had practically no effect upon yield, due possibly to delayed reaction on account of paucity of rainfall; and, finally, that inorganic toxins, as alkali, ferrous iron, and aluminum salts, are probably at present not directly responsible for lack of productivity.

## THE GREENHOUSE EXPERIMENTS

While field trials are generally considered as being the most reliable method of solving fertility problems, they are expensive and cumbersome, and, as has already been shown, should be executed over a period of years to allow for a fair average of climatic conditions. The quicker, less expensive, and more controllable pot experiment, as carried out in a well equipped greenhouse, is therefore often desirable. Coffey and Tuttle,<sup>7</sup> Wheeler, Brown and Hogensen,<sup>40</sup> and others have compared pot tests with field trials and have shown them to agree remarkably well where certain details of manipulation are followed. Furthermore, the ofttimes limiting factors of moisture and temperature may be so controlled in greenhouse work as to permit of more definite conclusions regarding possible plant food deficiencies. In the present work, this method of experimentation was especially adaptable, as frequent periodical soil-sampling was required.

The proper kind and quantity of fertilizer to apply depend as much upon the *total effect* produced within the soil solution as they do upon the element or elements directly supplied, for many of the changes induced may be indirect. For instance, sodium nitrate, so widely used as a source of available nitrogen, may so deflocculate a heavy soil as to render it non-productive. Much information is at present available in agricultural literature on the effects of additions of fertilizer salts and other chemical compounds upon the solubility of soil constituents. While a large portion of these data have been secured by subjecting the soils studied to artificial laboratory conditions, far removed from those actually obtaining in the field, nevertheless many of them have a sufficient bearing upon the present work to necessitate reviewing. More than seventy articles were read in this connection. However, as comprehensive references to the literature accompany the papers of Greaves and Carter,<sup>13</sup> Spurway,<sup>43</sup> and MacIntire,<sup>31</sup> it was thought best not to burden the reader with an extended review, very little of which could be directly compared with data to be subsequently presented, but rather to give a brief discussion of the work as a whole, noting the points in agreement as well as those at variance with the results hereafter given.

The chief impression made upon the reviewer of the literature within this field is the dissimilarity and often contradictory nature of results reported. For instance, certain writers have shown that additions of sodium nitrate to soils greatly enhance phosphate availability,

while a like number may easily be found who claim that sodium nitrate inhibits the solution of phosphates in soils. Similar differences of opinion exist regarding the effects of lime and gypsum upon the solubility of soil potash. An able discussion of such discrepancies, at least in so far as the effects of calcium carbonate and gypsum upon soil potassium are concerned, is given by Lipman and Gericke.<sup>29</sup> These writers attribute unlike and contradictory results to variations in the nature of the *mineral content* of the soils from different localities. The linkages binding potassium within the intricate silicate molecules doubtless vary greatly with different mineral complexes, the potassium being much more easily replaced by calcium, sodium, or other metallic ions in some instances than in others. As this might equally well apply to all the elements ordinarily met with in soils, one could hardly expect similar results to be obtained in all cases and for all elements. In fact, Lipman and Gericke,<sup>29</sup> Spurway,<sup>43</sup> Christie and Martin,<sup>6</sup> and many others give data which conclusively show that applications of the same salts in similar amounts react differently in different soils. Another factor which doubtless also plays a part is soil texture. The fine clays and clay loams presenting many times the internal surface found in the coarser silts and sands, should, and usually do, yield more material to solution. This is probably largely a mechanical factor.

Taking the recorded data on this subject by and large, the following statements seem to be justified in a majority of cases. The normal sulfates and chlorides of calcium, magnesium, sodium, and ammonium, may enhance the solubility of soil potassium and soil phosphorus, although the acid salts act much more strongly, especially in the case of the latter element. Nitrates act erratically, but we are fairly safe in saying that they usually slightly increase soil potash solubility, and exert little effect on soil phosphorus, although we know that the calcium phosphates are much more soluble in solutions of nitrates than are the iron and aluminum phosphates. The addition of calcium oxide usually increases potash solubility while the carbonate often has no direct effect. Phosphate solubility is usually depressed by lime applications, although this is not universally the case with quicklime, while the sulfates of the heavy metals often greatly increase it. Many writers have also shown that, under certain conditions, the soil bacteria, especially the nitrifiers, exert a decided solvent action upon the insoluble phosphates of both soils and fertilizers.

### Objects of the Pot Experiments

The objects of the pot experiments hereafter reported were: (1) so far as possible to eliminate climate, especially moisture, as a factor in crop production upon the soil studied, and to maintain throughout the growth period as nearly optimum conditions as possible; (2) to note the effects upon the growth of the pea plants, and upon the final yields of dried peas, of additions of the several fertilizers and soil amendments supplied; (3) to find whether or not such applications of chemical compounds influence the solubilities of the soil's constituents as manifested by periodical extractions of both planted and fallowed soils with distilled water; (4) to ascertain the effect of soluble salt applications upon the nodule formation of peas within this acid soil; (5) to ascertain whether or not soil toxins of any kind were inhibiting normal growth.

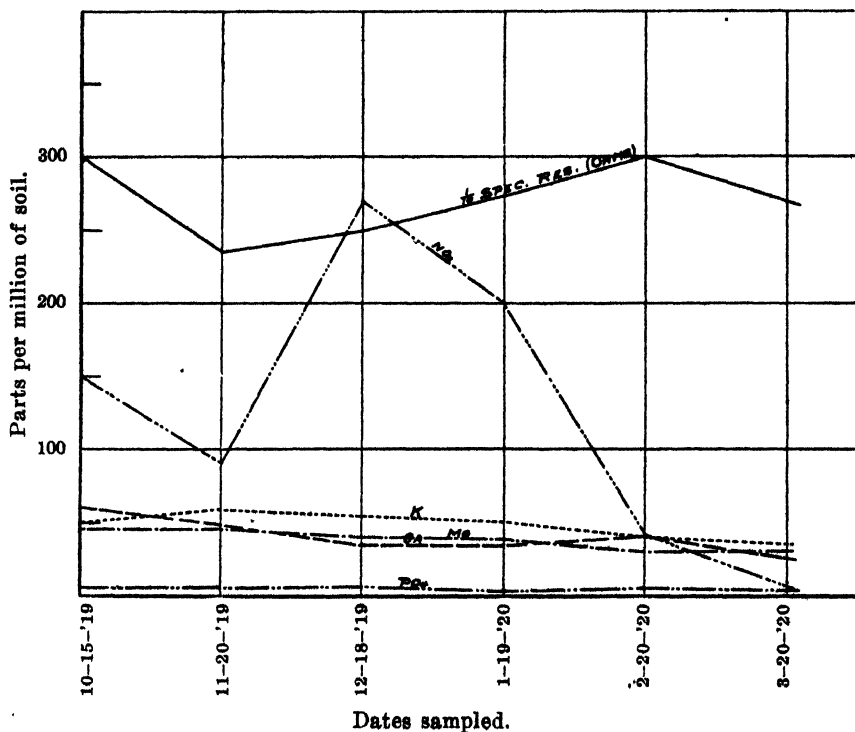


Fig. 5.—Water-soluble materials dissolved from cropped check pot soils (no fertilization).



### *Treatments Employed*

The experiments were carried out in a well regulated greenhouse. The pots used were 5-gallon glazed earthenware crocks about 12 inches in diameter and 11 inches deep. No holes were provided for drainage as it was desired that no soluble constituents be lost during the growth of the crops. The pots were weighed, and water added to optimum at

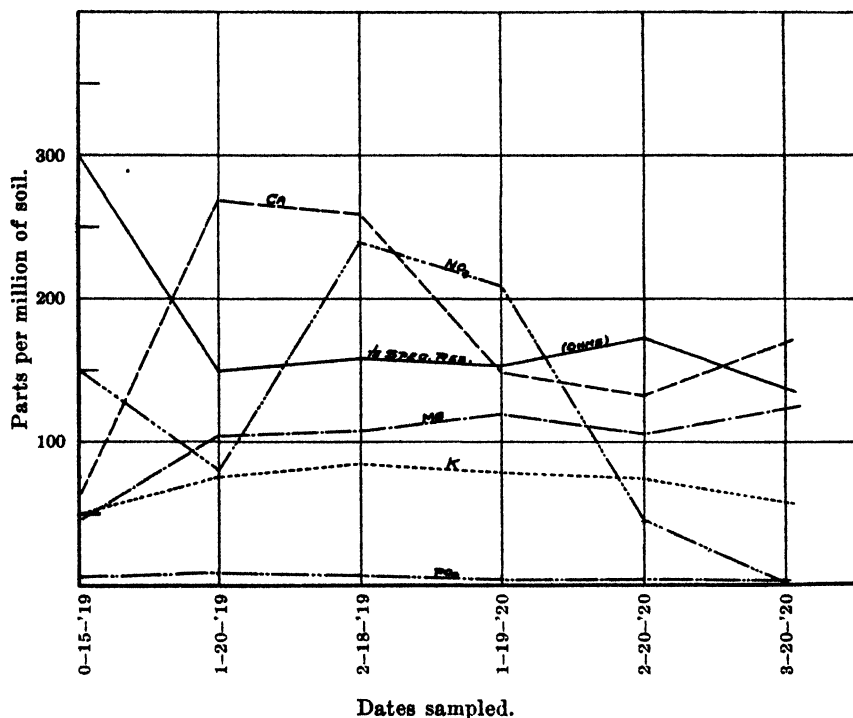


Fig. 6.—Water-soluble materials dissolved from cropped gypsum-treated pot soils.

each irrigation. The soil was procured during the month of August from the field plots above described, 6 two-bushel sacks of surface soil being taken from each of the 6 one-tenth acre plots. It was air-dry and dusty to a depth of approximately 6 inches. When received at the greenhouse, it was thoroughly mixed by being shoveled over five times and twice screened (one-fourth inch mesh) to remove the larger clods. Thirteen kilograms were then weighed into each of 64 pots, thus providing eight pots for each of the eight different treatments to be tested. The additions were made as follows:

Pots 1-8: Checks. No additions.

Pots 9-16: Gypsum at the rate of 1 T. per acre (20 g. per pot).

Pots 17-24:  $\text{CaCO}_3$  at the rate of 8 T. per acre (160 g. per pot).

Pots 25-32: Superphosphate at the rate of 1 T. per acre (20 g. per pot).

Pots 33-40:  $\text{NaNO}_3$  at the rate of 500 lbs. per acre (5 g. per pot).

Pots 41-48:  $\text{K}_2\text{SO}_4$  at the rate of 500 lbs. per acre (5 g. per pot).

Pots 49-56: Super. (1 T. per a.) and  $\text{K}_2\text{SO}_4$  (500 lbs. per a.).

Pots 57-64: Super. (1 T. per a.) and  $\text{CaCO}_3$  (8 T. per a.).

As will be observed, the applications here made were in all cases consistent with good field practice. The amounts of salts (dry) as indicated were thoroughly mixed into the surface six inches of soil

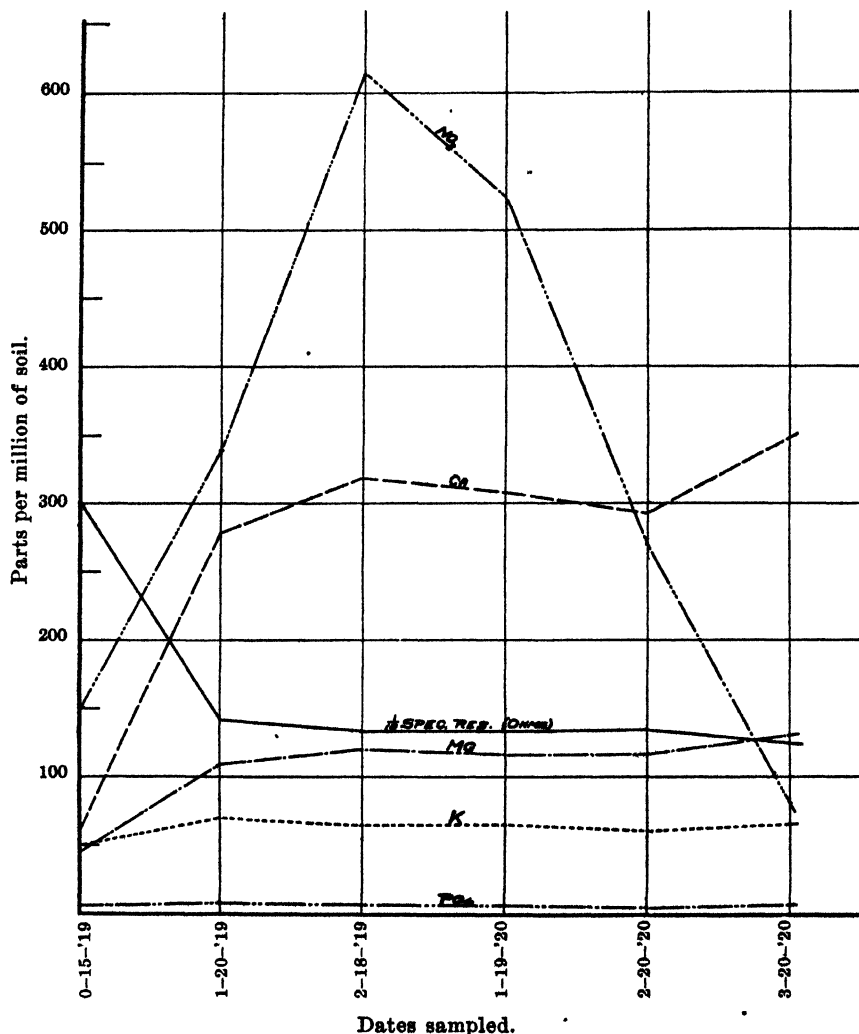


Fig. 7.—Water-soluble materials dissolved from cropped calcium-carbonate-treated pot soils.

in each pot, after which the soils were settled into place by an irrigation calculated to be optimum (one-half total moisture holding capacity). The pots were then allowed to stand for one week before planting. The salts applied were "Bakers C. P. Analyzed Chemicals" in all cases except the superphosphate, which was the same as that used in the field plot experiments. At the same time, a set of six pots of the soil, which were to be kept fallow (no crop) were set up. The first six, single-salt treatments only were here employed. These fallowed pots were subsequently treated in exactly the same way as the cropped pots.

The peas (Horseford's Market Garden variety) were sown on November 10, 1919; eight uniform seeds to the pot. A good stand was obtained. When the plants were about three inches high, they were thinned to four per pot. When 6 to 8 inches high, the peas were trellised, using split laths and string. The floor plan of the greenhouse indicating the arrangement of the benches and the pots is shown in figure 2. As the plants grew taller and shading was evident at certain periods during the day, the practice of changing the pots from one bench to the other each week at the time of irrigation was adopted.

As one of the objects of the work was to ascertain the effects of the several salt applications upon soil-mineral solubility, at approximately four-week intervals samples of the cropped soils were withdrawn from the pots and analyses made in accordance with the detailed methods previously given. The results of this work appear in Table VIII and, for convenience, are graphically shown in figures 5 to 19. It had been planned also to extract and analyze the similarly treated fallowed soils each month, but, as the two series will be shown to be hardly comparable, and as time for this large amount of analytical work was wanting, the uncropped soils were analyzed but four times during the experiment (during October, November, January, and April). The results of these analyses appear in Table IX.

After thinning, and when the plants had reached a height of 6 or 7 inches, some trouble was experienced with mice. In eight or ten of the pots, one or two of the plants were destroyed. This difficulty was quickly overcome, but not before some damage had been done. For this reason, in Table X, only six pots (out of the eight of each treatment) giving the highest yields per pot, and having four plants each, have been used in computing statistically the final yields obtained, although the yields in all of the pots are given.\*

\* As stated in the table, a star (\*) marks those figures omitted from the averages. The data, when plotted, gave uniform frequency curves.

TABLE VIII  
PERIODIC DETERMINATIONS ON CROPPED, POT SOILS

Treatment Number	Dates of Sampling Soils					
	10-15-19	11-20-19	12-18-19	1-19-20	2-20-20	8-20-20
Acidity expressed in $P_H$						
1	4.46	4.51	4.50	4.48	4.46	4.71
2	4.46	4.55	4.58	4.50	4.51	4.73
3	4.46	7.20	7.20	7.39	7.34	7.25
4	4.46	4.67	4.62	4.62	4.60	4.71
5	4.46	4.67	4.72	4.53	4.63	4.88
6	4.46	4.67	4.68	4.67	4.67	4.88
7	4.46	4.63	4.63	4.63	4.65	4.80
8	4.46	7.30	7.33	7.46	7.42	7.33
Specific Resistance in Ohms						
1	3,000	2,381	2,515	2,752	3,053	2,726
2	3,000	1,498	1,568	1,517	1,728	1,402
3	3,000	1,420	1,331	1,325	1,357	1,261
4	3,000	1,280	1,568	1,856	2,022	1,587
5	3,000	2,112	2,029	2,131	2,374	2,302
6	3,000	2,131	2,054	2,400	2,509	2,118
7	3,000	1,286	1,472	1,523	1,702	1,382
8	3,000	1,171	1,133	998	1,088	1,018
Calcium-ion, parts per million						
1	60	49	35	34	41	25
2	60	269	262	150	133	169
3	60	279	319	309	295	350
4	60	131	136	104	88	128
5	60	59	59	50	43	48
6	60	169	71	55	52	56
7	60	160	150	121	102	169
8	60	387	430	428	434	500
Magnesium-ion, parts per million						
1	45	46	40	37	30	29
2	45	105	109	120	107	125
3	45	110	121	117	116	130
4	45	110	106	79	78	102
5	45	55	53	48	46	38
6	45	70	67	56	48	55
7	45	133	125	98	86	127
8	45	145	147	144	150	154
Potassium-ion, parts per million						
1	50	58	54	50	39	35
2	50	75	84	80	75	60
3	50	69	64	65	60	63
4	50	87	81	68	51	61
5	50	65	66	58	41	37
6	50	88	86	75	58	63
7	50	117	117	97	70	92
8	50	75	69	74	56	65

TABLE VIII—(Continued)

Treatment Number	Dates of Sampling Soils					
	10-15-19	11-20-19	12-18-19	1-19-20	2-20-20	3-20-20
Phosphate-ion, parts per million						
1	5.2	3.7	4.5	2.3	3.5	2.1
2	5.2	6.2	5.6	4.0	4.0	3.4
3	5.2	6.2	4.7	4.5	4.1	5.4
4	5.2	8.5	6.8	8.4	7.2	4.1
5	5.2	5.6	5.9	4.1	4.3	2.3
6	5.2	5.6	5.6	4.7	4.5	2.0
7	5.2	7.4	7.0	7.4	6.9	3.2
8	5.2	7.6	6.7	7.8	7.0	5.6
Nitrate-ion, parts per million						
1	150	89	267	204	35	5
2	150	80	239	213	44	0
3	150	338	621	532	266	84
4	150	156	221	177	27	0
5	150	488	488	400	177	30
6	150	178	266	177	44	5
7	150	156	266	177	40	7
8	150	196	485	443	266	156

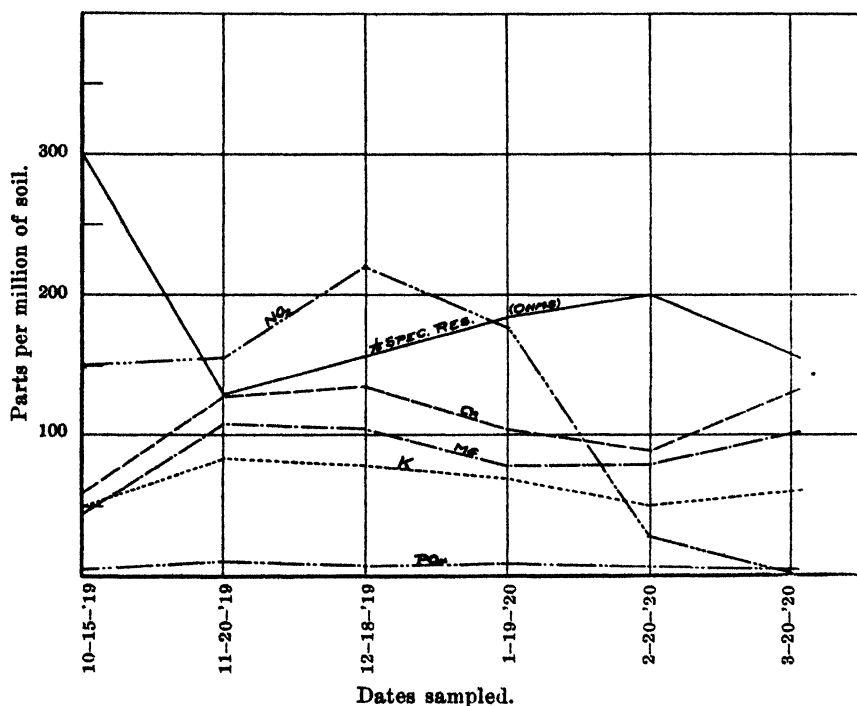


Fig. 8.—Water-soluble materials dissolved from cropped superphosphate-treated pot soils.

TABLE IX  
PERIODIC DETERMINATIONS ON FALLOWED, POT SOILS

Treatment Number	Dates of Sampling Soils			
	10-15-19	11-20-19	1-25-20	4-1-20
Acidity expressed in $P_H$				
1	4.46	4.50	4.43	4.32
2	4.46	4.55	4.50	4.38
3	4.46	7.20	7.17	7.17
4	4.46	4.67	4.46	4.46
5	4.46	4.67	4.45	4.43
6	4.46	4.67	4.40	4.38
Specific Resistance in Ohms				
1	3,000	2,380	lost	2,048
2	3,000	1,498	lost	1,338
3	3,000	1,420	lost	1,011
4	3,000	1,280	lost	2,180
5	3,000	2,112	lost	1,587
6	3,000	2,131	lost	1,754
Calcium-ion, parts per million				
1	60	49	72	81
2	60	269	250	209
3	60	279	514	512
4	60	131	112	99
5	60	59	104	100
6	60	169	128	104
Magnesium-ion, parts per million				
1	45	46	67	80
2	45	105	147	161
3	45	110	144	157
4	45	110	105	105
5	45	55	86	96
6	45	70	67	98
Potassium-ion, parts per million				
1	50	58	58	59
2	50	75	86	84
3	50	69	65	59
4	50	87	81	73
5	50	65	68	72
6	50	88	86	96
Phosphate-ion, parts per million				
1	5.2	3.7	3.7	2.3
2	5.2	6.2	4.1	2.4
3	5.2	6.2	4.5	4.3
4	5.2	8.5	8.4	8.8
5	5.2	5.6	5.6	4.1
6	5.2	5.6	4.7	2.3
Nitrate-ion, parts per million				
1	150	89	400	575
2	150	80	756	708
3	150	338	1,264	1,106
4	150	156	550	496
5	150	488	421	940
6	150	178	355	575

### Crop Yields

The effects of the several soil treatments upon crop yields will first be considered. Table X presents this data while a chart showing graphically the comparative yields of both total dry matter and cured peas appears in figure 20. The "plus or minus" variability factors as shown in figure 20 are obtained by multiplying the "probable error of the mean" in each case (Table X) by three, thus insuring practically a thirty to one chance that, in case of repetition, the new average yields found will fall within these limits. Those figures also help us in determining approximately\* whether or not significant differences in yields are shown between treatments.†

The most notable fact impressed upon one who has carefully followed both the field and the greenhouse experiments is that the plants grown in the greenhouse under nearly ideal climatic conditions grew to at least twice the size and probably, plant for plant, produced nearly twice as many peas as those grown in the field at the Marin Meadows Ranch. Although the crop on the field plots was above the average, the individual plants were small in comparison with any (checks included) grown inside. That water has been one of the important limiting factors in the field during the past season can hardly be questioned.

The second point to be noticed is the comparatively *small* increase over the check pots due to any of the fertilizer applications. One would certainly expect a soil so low in soluble phosphorous or so acid in reaction to respond *greatly* to applications of either soluble phosphates or lime, and certainly where both were used. But no such large increases are apparent. It is true that enhanced yields follow the application of

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\* The *exact* method of determining whether or not a difference is significant is to take the square root of the sum of the squares of the two probable errors of the two means, multiply the resulting figure by 3 and note whether or not the product is larger or smaller than the subtracted difference between the two means. In case it is smaller, it is safe to conclude that the difference between the two means is significant, taking a 30 to 1 chance of securing a similar result upon repetition. For example, let us compare the average yield of total dry matter secured in the check pots with that where gypsum was applied, and find whether or not gypsum *actually* increased yields:

$$59.5 \pm 0.9 = \text{mean of gypsum pots.}$$

$$51.4 \pm 1.3 = \text{mean of check pots.}$$

$$8.1 \pm \sqrt{0.9^2 + 1.3^2} \times 3$$

$$= 8.1 \pm \sqrt{2.50} \times 3$$

$$= 8.1 \pm 4.6$$

As 4.6 is much less than 8.1, we are safe in concluding that there is a significant difference shown here between the means, and that the application of gypsum did actually slightly increase yields.

† The scale to the left of fig. 20 should be used in this connection.

TABLE X  
YIELDS OF PEAS IN GREENHOUSE POT EXPERIMENT

Treatment No. 1 (Checks)			
Pot Number	Total Dry Weights	Peas in Pods	Shelled Peas
1	53.6	37.1	29.7
2	45.1*	28.4*	21.1*
3	58.6	37.7	30.9
4	53.6	34.9	28.9
5	50.7*	38.6*	31.5*
6	51.0	34.5	28.0
7	44.2	33.9	26.1
8	47.2	34.1	28.0
Mean	51.4 $\pm$ 1.3	35.4 $\pm$ 0.4	28.6 $\pm$ 0.4
Std. Dev.	4.7 $\pm$ 1.4	1.5 $\pm$ 0.4	1.5 $\pm$ 0.4
C. V.	9.2 $\pm$ 1.8%	4.2 $\pm$ 0.8%	5.2 $\pm$ 1.0%
P. E.	$\pm$ 3.2	$\pm$ 1.0	$\pm$ 1.0
Treatment No. 2 (Gypsum)			
Pot Number	Total Dry Weights	Peas in Pods	Shelled Peas
9	53.7*	34.3*	25.5*
10	55.8	37.3	29.5
11	64.3	37.6	29.8
12	62.9	35.6	28.4
13	59.0	33.0	29.8
14	58.1	36.7	29.7
15	56.6	33.9	26.9
16	56.9*	34.0*	26.4*
Mean	59.5 $\pm$ 0.9	35.7 $\pm$ 0.4	29.0 $\pm$ 0.3
Std. Dev.	3.1 $\pm$ 0.9	1.7 $\pm$ 0.5	1.0 $\pm$ 0.3
C. V.	5.2 $\pm$ 1.0%	4.8 $\pm$ 0.9%	3.4 $\pm$ 0.7%
P. E.	$\pm$ 2.1	$\pm$ 1.1	$\pm$ 0.7
Treatment No. 3 (Calcium carbonate)			
Pot Number	Total Dry Weights	Peas in Pods	Shelled Peas
17	68.7*	38.6*	30.6*
18	70.5	42.9	34.6
19	70.6	41.2	33.6
20	70.2	42.8	36.5
21	67.0	40.9	33.3
22	65.0	37.2	31.6
23	69.1	44.2	36.6
24	61.3*	36.5*	29.2*
Mean	68.7 $\pm$ 0.6	41.5 $\pm$ 0.6	34.4 $\pm$ 0.5
Std. Dev.	2.1 $\pm$ 0.6	2.2 $\pm$ 0.6	1.8 $\pm$ 0.5
C. V.	3.1 $\pm$ 0.6%	5.3 $\pm$ 1.0%	5.2 $\pm$ 1.0%
P. E.	$\pm$ 1.4	$\pm$ 1.5	$\pm$ 1.2
Treatment No. 4 (Superphosphate of lime)			
Pot Number	Total Dry Weights	Peas in Pods	Shelled Peas
25	58.5	35.8	29.7
26	60.4	36.4	29.7
27	56.3*	34.2*	27.4*
28	68.5	40.8	32.6

\* Omitted from average.



TABLE X—(Continued)

Pot Number	Total Dry Weights	Peas in Pods	Shelled Peas
29	61.8	36.5	29.9
30	68.6	40.0	32.0
31	69.1	37.8	30.5
32	60.4*	33.4*	27.8*
Mean	64.5±1.2	38.0±0.5	30.7±0.3
Std. Dev.	4.4±1.3	1.9±0.5	1.1±0.3
C. V.	6.8±1.3%	5.0±0.9%	3.6±0.6%
P. E.	±3.0	±1.3	±0.7

## Treatment No. 5 (Sodium nitrate)

Pot Number	Total Dry Weights	Peas in Pods	Shelled Peas
33	59.2	36.0	28.7
34	59.9*	32.1*	24.3*
35	60.1	36.9	30.0
36	55.9	33.0	26.5
37	60.5	35.2	29.1
38	54.7	33.4	27.0
39	53.1*	29.0*	23.2*
40	55.0	30.2	25.0
Mean	57.6±0.7	34.1±0.6	27.7±0.5
Std. Dev.	2.4±0.7	2.2±0.6	1.7±0.5
C. V.	4.2±0.8%	6.4±1.2%	6.1±1.1%
P. E.	±1.6	±1.5	±1.2

## Treatment No. 6 (Potassium sulfate)

Pot Number	Total Dry Weights	Peas in Pods	Shelled Peas
41	52.8	33.1	26.7
42	54.8	31.4	24.8
43	50.0*	29.4*	23.6*
44	50.6	33.0	26.2
45	56.6	34.4	27.7
46	52.3	31.8	25.9
47	58.6	35.6	30.6
48	54.8*	29.6*	24.5*
Mean	54.3±0.7	33.2±0.4	27.0±0.5
Std. Dev.	2.7±0.8	1.4±0.4	1.8±0.5
C. V.	5.0±0.9%	4.2±0.8%	6.6±1.2%
P. E.	±1.8	±0.9	±1.2

Treatment No. 7 (Super. plus  $K_2SO_4$ )

Pot Number	Total Dry Weights	Peas in Pods	Shelled Peas
49	49.7*	28.3*	23.0*
50	47.8	30.1	25.5
51	47.4*	23.3*	18.6*
52	53.5	31.3	25.2
53	57.0	33.1	29.1
54	51.6	31.2	26.2
55	56.7	33.2	27.4
56	57.4	34.8	29.1
Mean	54.0±1.0	32.3±0.4	27.1±0.4
Std. Dev.	3.5±1.0	1.6±0.5	1.6±0.5
C. V.	6.5±1.2%	4.9±1.0%	5.9±1.2%
P. E.	±2.4	±1.1	±1.1

\* Omitted from average.

TABLE X—(Continued)  
Treatment No. 8 (Super. plus  $\text{CaCO}_3$ )

Pot Number	Total Dry Weights	Peas in Pods	Shelled Peas
57	64.7	41.0	34.3
58	54.5*	33.0*	27.9*
59	62.8	38.0	32.4
60	55.9	35.2	30.4
61	54.9*	33.0*	28.2*
62	64.6	42.7	35.7
63	60.6	39.1	33.2
64	71.9	48.2	41.1
Mean	$63.4 \pm 1.3$	$40.7 \pm 1.1$	$34.5 \pm 0.9$
Std. Dev.	$4.8 \pm 1.4$	$4.1 \pm 1.2$	$3.3 \pm 1.0$
C. V.	$7.5 \pm 1.4\%$	$10.1 \pm 1.8\%$	$9.6 \pm 1.8\%$
P. E.	$\pm 3.2$	$\pm 2.8$	$\pm 2.12$

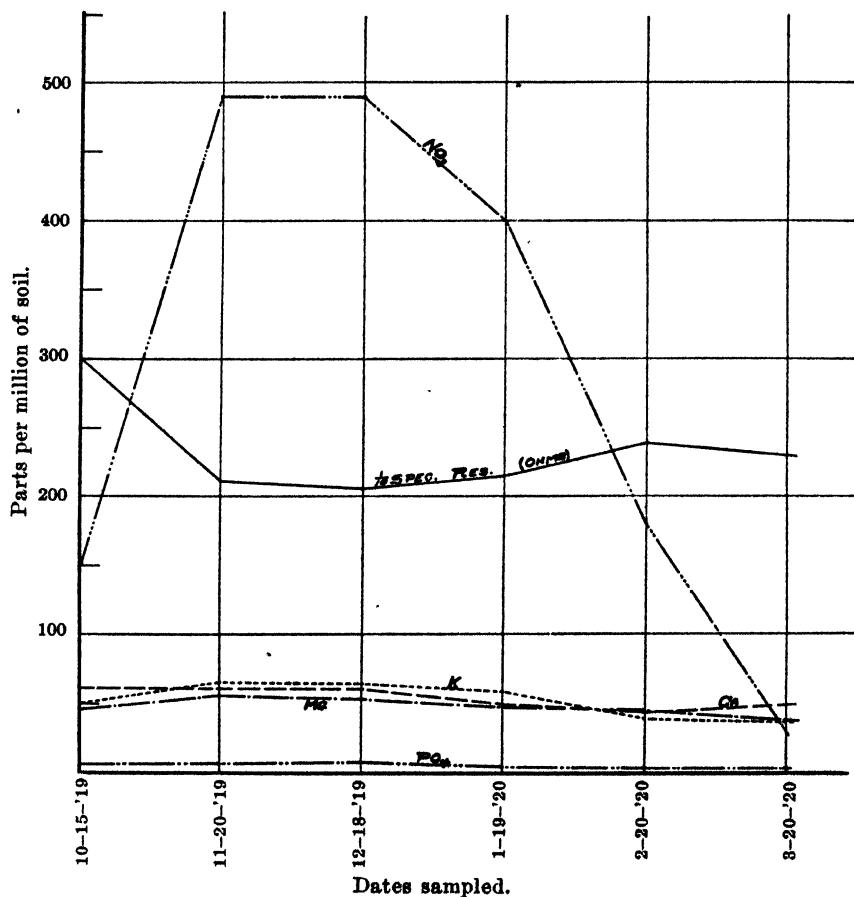


Fig. 9.—Water-soluble materials dissolved from cropped sodium-nitrate-treated pot soils.

\* Omitted from average.

certain of these compounds, but they amount to relatively little. Let us observe the chart showing comparative yields (fig. 20), first taking up "Total Dry Weights" produced. Treatment No. 1 (checks) produced lower yields than did any of the others, yet brief computations show that the differences between the checks and treatments 6 ( $K_2SO_4$ ) and 7 ( $K_2SO_4$  + superphosphate) are not significant, while the real difference between the checks and 5 ( $NaNO_3$ ) is so slight (less than 2

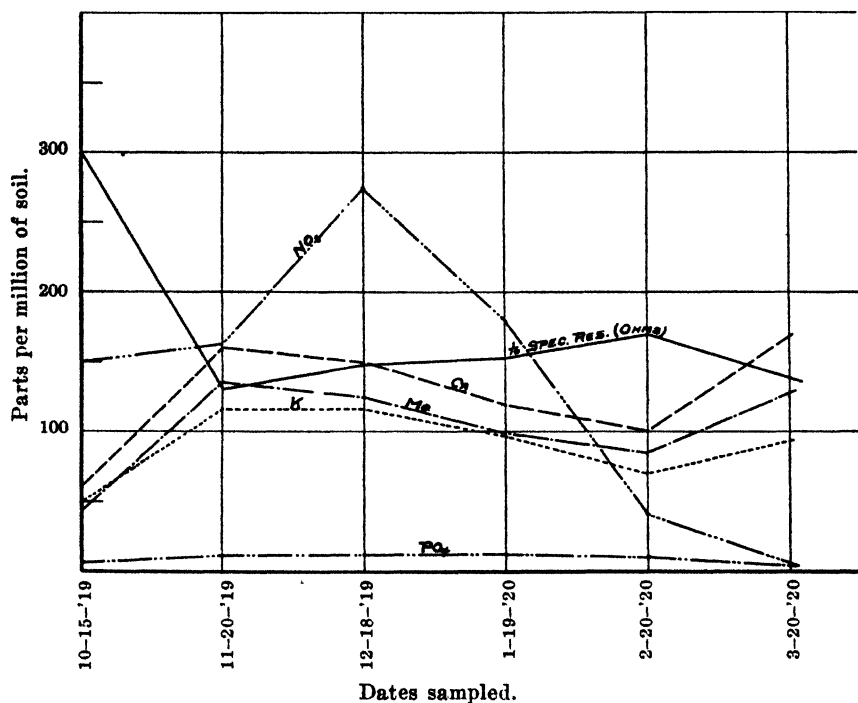


Fig. 10.—Water-soluble materials dissolved from cropped potassium-sulfate-treated pot soils.

grams) as to be well-nigh negligible. We are, however, justified in stating that liming to neutrality did actually increase the yields of peas in the greenhouse over the checks by nearly 35%; that applications of superphosphate, at the rate of one ton per acre, gave an increase of approximately 28%; that the same amounts of superphosphate and  $CaCO_3$  when used together increased yields no more than did either when added separately; and that gypsum at the rate of one ton per acre was about one-half as effective as  $CaCO_3$  when the latter was used in sufficient quantities to neutralize soil acidity (8 tons per acre). It will be recalled that, in the field, superphosphate alone

gave increased yields, while calcium carbonate, added to neutrality, had little effect. The comparative solubilities of the two, water being limited in the field, may well account for these differences. The final yields of dry matter obtained, however, do not show the comparative rates of growth nor do they reflect the conditions of the plants at the various monthly periods of sampling. During the entire experiment

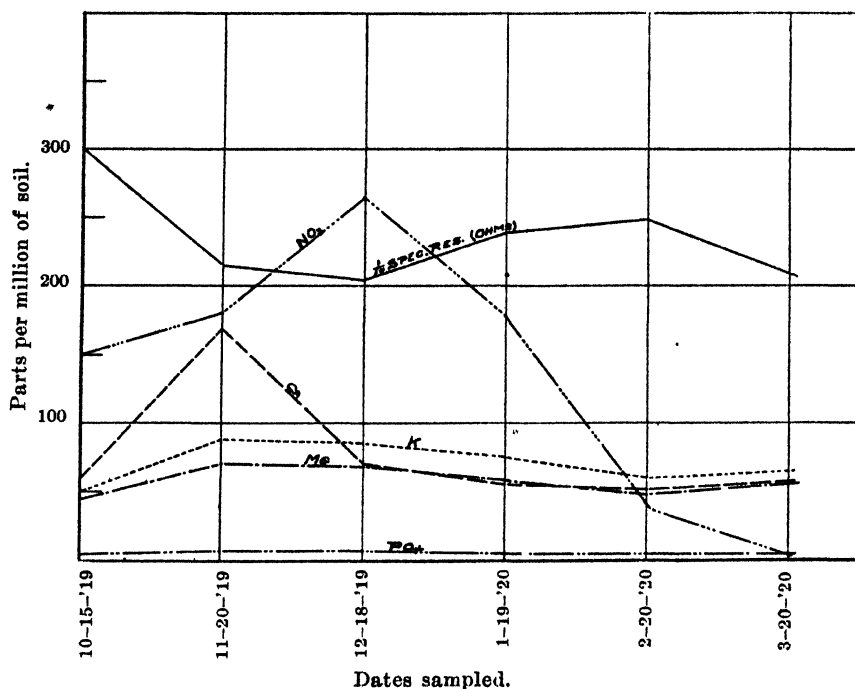


Fig. 11.—Water-soluble materials dissolved from cropped pot soils receiving both superphosphate and potassium sulfate.

the phosphate treated plants were apparently far ahead of all others in size, color, and general condition. They bloomed and set pods at least a week before the other treatments and matured ten days earlier than the others. The nitrate treated plants started well but soon fell behind. The lime treated plants made a slow, steady growth from the start, and, as will be seen, gave maximum yields both of total dry matter produced and of dry peas. Potassium sulfate wherever applied seemed at all times to retard growth. This may be due to the considerable quantity of sulfate-ion added, as the soil already carried nearly one-tenth of one per cent sodium sulfate. Gypsum also at first impeded growth. Figure 21 gives one a good

idea of the plants when the pods were setting (about one month before harvesting). It serves to compare the several treatments, an average pot from each series being taken in each case.

Let us now consider the comparative weights of dried, shelled peas produced by the different salt applications (see fig. 20). The results

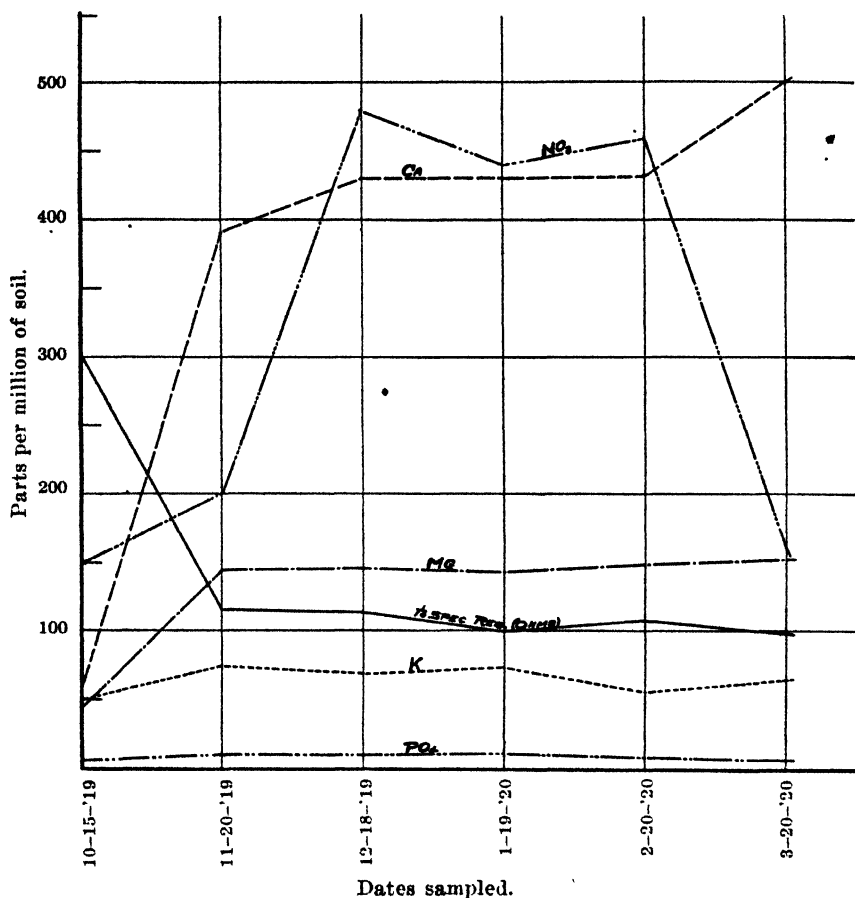


Fig. 12.—Water-soluble materials dissolved from cropped pot soils receiving both superphosphate and calcium carbonate.

are slightly different from those considered above. The calcium carbonate and the superphosphate treatments alone produced significant increases, while treatments of sodium nitrate and of potassium sulfate apparently decreased the yields, although the decreases are hardly significant. One can see from the data presented that liming to neutrality is the treatment *par excellence* for this soil type where

optimum moisture and temperature conditions obtain. The use of superphosphate without lime increases the yield of peas but 6%, while the addition of lime alone gives us an 18% increase over the check pots. In treatment 8, where both lime and superphosphate are applied, the yield is the same as where lime alone is used. The soil solubility studies to follow explain this point by showing that the calcium carbonate application renders soluble such amounts of soil

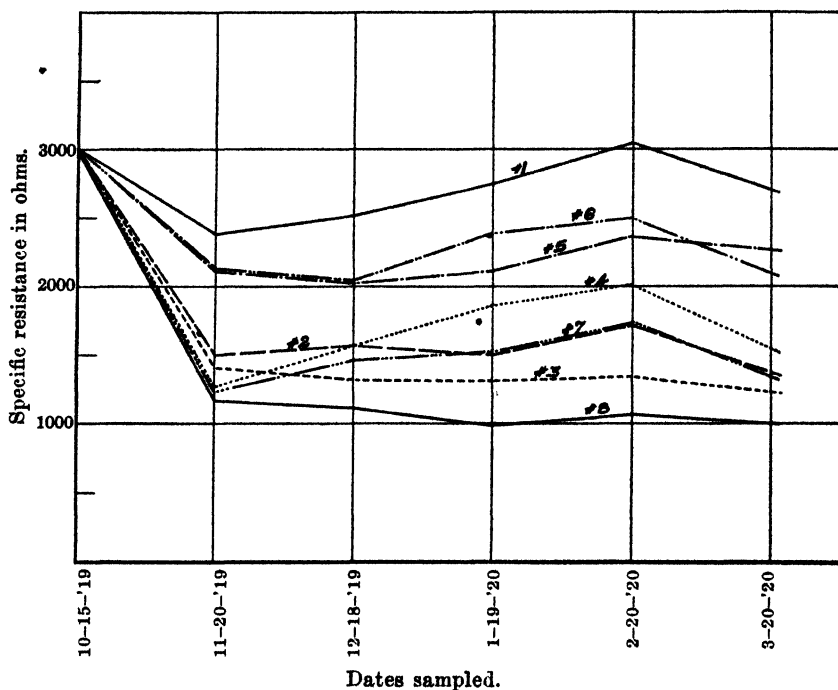


Fig. 13.—Effects of different treatments on specific resistances of water extracts.

phosphorus that still further applications of this element are unnecessary (see fig. 18, curves 4 and 8). At no time during the development of the plants in the greenhouse was the presence of soil toxins in any way manifested. Certain other points of interest regarding comparative yields will be noted later in connection with the soil solubility studies.

Immediately after harvesting (on April 10), the soils were carefully removed from the pots and the roots examined for nodule production. Previous experience in the field had shown this soil to be well supplied with the strain of *B. radicicola* capable of producing

nodules on pea roots. There was but little variation between the individual pots of the same treatment, the following general statements applying in each case:

Treatment No. 1: A few large nodules. Several small ones per pot.

Treatment No. 2: Similar to No. 1. Possibly a few more small nodules.

Treatment No. 3: A *very few* small nodules. But slightly better than No. 5.

Treatment No. 4: Best of all treatments. Large numbers of nodules both large and small. Many near bottom of pots.

Treatment No. 5: No nodules found.

Treatment No. 6: Very large numbers of small nodules. No large clusters.

Treatment No. 7: Large numbers of nodules, chiefly small, although a few large clusters were noted. Almost as good as No. 4.

Treatment No. 8: Very few small nodules. Similar to No. 3.

At first thought it might seem incredible that such an acid soil ( $P_H$  4.5) could harbor viable strains of *B. radiculicola*. Fred and Davenport,<sup>12</sup> however, in a series of very carefully controlled experiments, have given data to show that certain of the *B. radiculicola* group are very resistant to acidity. All of the species apparently may withstand a reaction, in liquid media, of  $P_H$  5. They state:

The nodule bacteria from different plants behave very differently toward acid. The legume bacteria may be divided into groups about as follows:

1. Critical  $P_H$ —4.9 Alfalfa and sweet clover.
2. Critical  $P_H$ —4.7 Peas and vetch.
3. Critical  $P_H$ —4.2 Clover and common beans.
4. Critical  $P_H$ —3.3 Soy and velvet beans.
5. Critical  $P_H$ —3.15 Lupines.

The evidence supports the conclusions that a correlation exists between the acid resistance of the bacteria and the acid resistance of the higher plant.

Since their bacteriological work was carried on in solution cultures, it may not be directly comparable with soil conditions, although it should be added that beans on the soil under experiment grow better than do either peas or alfalfa. This sequence would be expected from the data above presented.

Upon further observance of the effect of the soil treatments on nodule formation, we note that where nitrates were added, no nodules appeared, and, contrary to expectation, where  $CaCO_3$  was applied to neutrality but *very few* small nodules were found. The reason is probably the same in both cases (see fig. 19, curves 3, 5, and 8),

namely, a superabundance of nitrate-nitrogen. Many articles are extant showing the depressing tendency of large amounts of soil nitrates on nodule formation. Superphosphate has often been observed to enhance nodule production. Our studies are in agreement with these findings. Potash and gypsum treatments but slightly enhanced nodule formation.

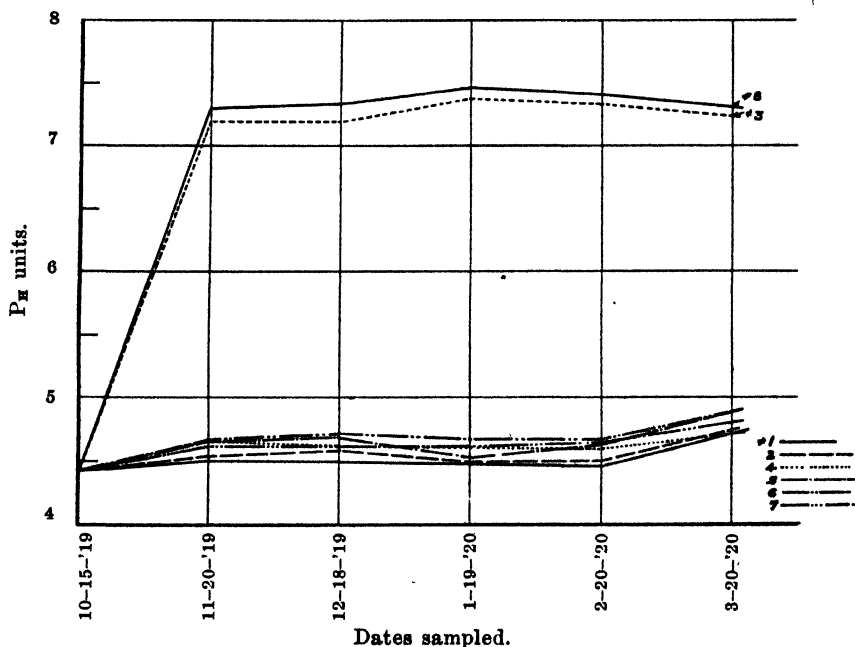


Fig. 14.—Effects of different treatments on hydrogen-ion concentrations of soils.

### Soil Extraction Studies

It remains for us to discuss the interesting data secured by periodically extracting the differently treated soils with distilled water and noting the effects of both the fertilizer applications and the growing plants upon the concentration of soil solutes. The importance of knowledge of both the direct and the indirect effects of fertilizer chemicals upon soils has been briefly pointed out in the introduction to these studies. Stewart<sup>44</sup> has shown very fully the effects of a growing, unfertilized crop of barley upon the concentration of the soil solution. During the first six to eight weeks, a considerable increase in soluble nutrients was usually observed. This was especially true of nitrates. The growing crop then began to draw heavily upon this store with the



result that in most soils a gradual decrease in concentration was noted. He found that fertile soils were sometimes exceptions to this rule, the concentrations remaining practically constant throughout the entire growth period. The cause of this was pointed out as being doubtless due to the abilities of very fertile soils to renew soluble materials as

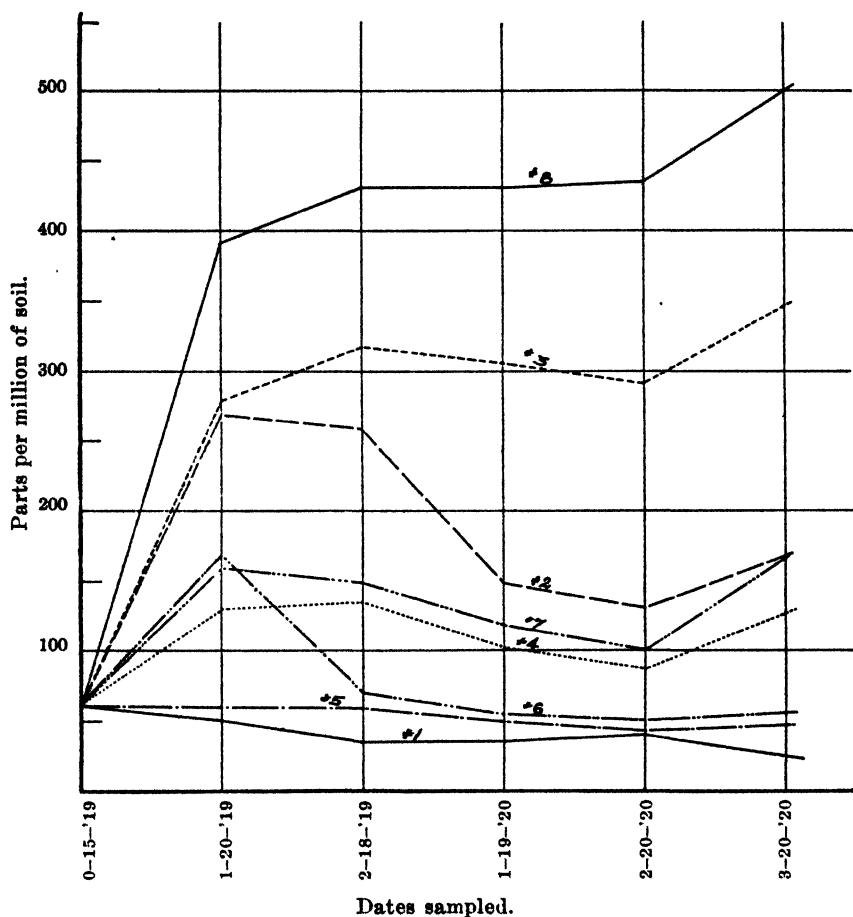


Fig. 15.—Effects of different treatments on calcium-ion solubility.

rapidly as they were withdrawn. Hoagland,<sup>21</sup> Millar,<sup>24</sup> and McCool and Millar<sup>22</sup> have shown that the solutes in the soil solution vary greatly at different periods and are materially affected by the growth of plants.

In the present investigation such effects are well shown in the curves presented in fig. 5. Upon the abscissae have been plotted the dates of sampling, while upon the ordinates appear the concentrations

of the various ions in parts per million of dry soil. Table VIII lists the data from which these curves were constructed. The graphs represent results secured from the eight check pots which received no fertilizing materials. Only slight differences in water-soluble potassium, magnesium, calcium, and phosphorus are here shown at the different sampling dates, while during the last two months a gradually declining tendency is noticed. The absolute amounts of these elements,

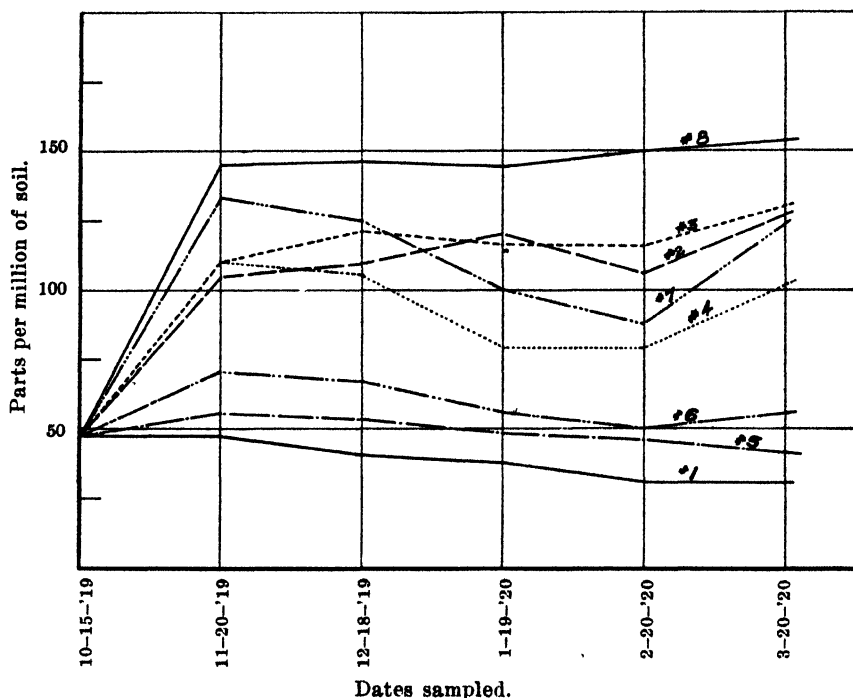


Fig. 16.—Effects of different treatments on magnesium-ion solubility.

present in a readily soluble form, are above those usually secured from the poor soils reported by Stewart, with the exception of  $\text{PO}_4$ -ion, the amount of which is unusually low. The nitrate-ion gradually increased in quantity during the first two months of growth, then fell off until, at the time of crop maturity, none remained. The results of the chemical work as carried out on the untreated field plot soils (fig. 3) are in close agreement with the greenhouse checks, except that nitrates, in the field, at no time equal the large quantities at first present in the irrigated pot soils.

Let us now briefly consider the effects of the several treatments upon the solubilities of the constituents of this clay loam soil. The

check pots, which received no additions, will be taken as the basis for comparison. Both the cropped and fallowed soils will be discussed.

The specific resistances of the soil extracts were always determined and are of importance in that they give us, in such dilute solutions, a comparative measure of total soluble salt concentrations. One-tenth of the specific resistance, in ohms, is shown by the solid lines in the graphs. It will be seen that these vary inversely with the concentra-

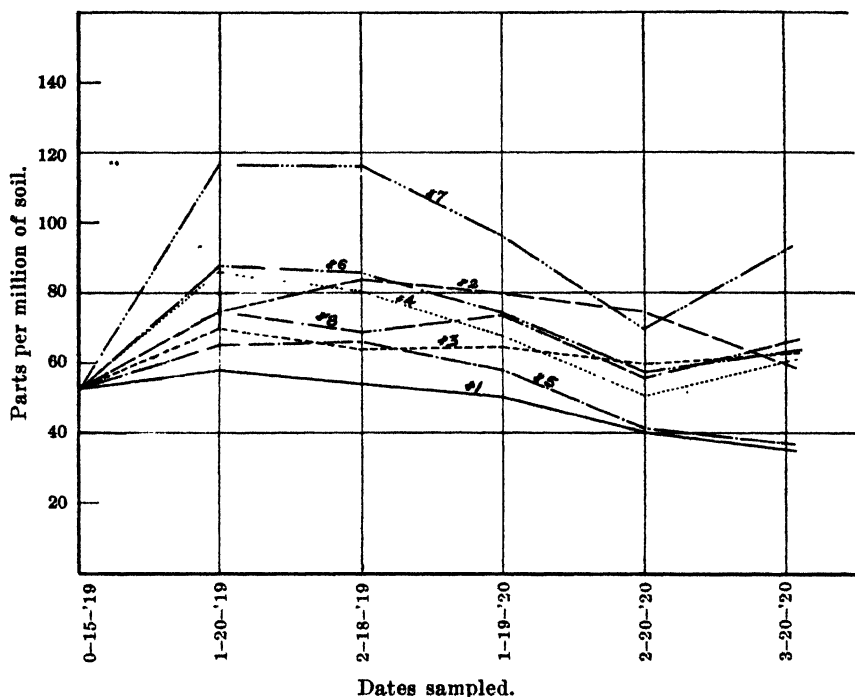


Fig. 17.—Effects of different treatments on potassium-ion solubility.

tion of soil solutes and that a general relationship exists between water-soluble salts and crop production.

Gypsum at the rate of one ton per acre was applied to the pots in treatment 2 (see fig. 6). Contrary to many general statements in the literature, nitrate production has not here been appreciably affected. The amount of water-soluble magnesium, however, has been increased almost threefold, while the amount of soluble potassium has been doubled under a rapidly growing crop. The amount of phosphate-ion was slightly increased at first, but soon fell to the level of the checks. Calcium, as would be expected, remained at a high level throughout the experiment. Sulfates, occasionally determined but not shown in

the graphs, were highest in the gypsum treatments. In the fallowed soils (Table IX), the results were much the same, except that the high level of concentration occurred a little later for all of the ions except magnesium. Here there was a gradual progressive increase. The actual concentrations of water-soluble compounds in the fallowed soils in all cases reached much higher levels than were reached in the cropped pots.

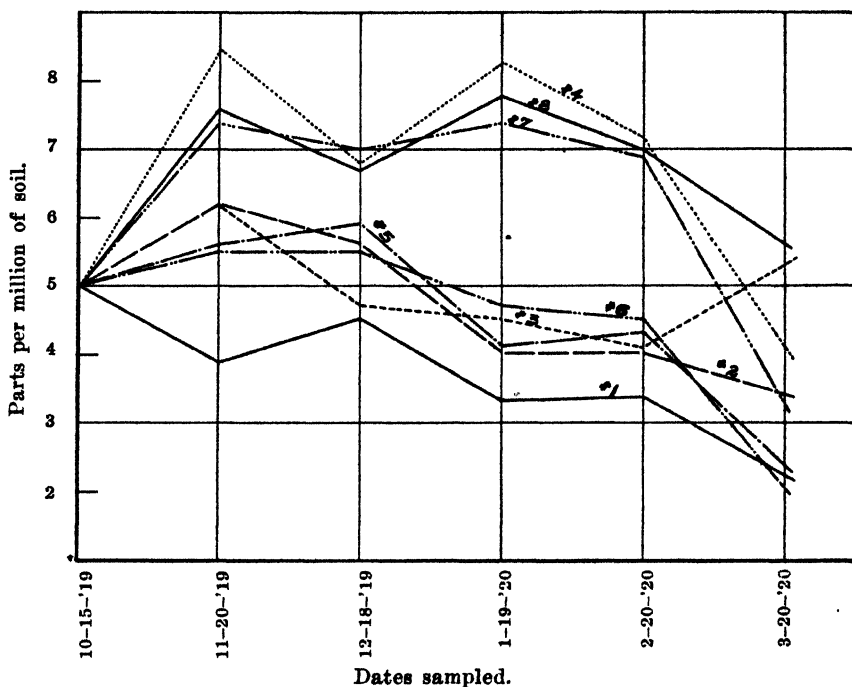


Fig. 18.—Effects of different treatments on phosphate-ion solubility.

The  $\text{CaCO}_3$  applications increased nitrate production (from soil nitrogen) at least threefold throughout the growing period (see fig. 7). The same is true of the production of soluble magnesium. Soluble potassium and phosphorus are each increased by approximately 50 per cent. Calcium, in a readily water-soluble form, has been increased from an average of 40 p. p. m. in the checks to over 300 p. p. m. in the lime-treated pots. The specific resistance of the soil extract is very low throughout. With the exception of nitrate (and this tendency is also shown in the uncropped soil) the lime treatment not only *maintains* the concentrations of the several ions during the period of vigorous absorption of solutes, but actually increases the rate of solubility of

minerals over and above crop demands, for we see that, on March 20, at the time of maturity there is shown a slight rise in the phosphorus, potassium, magnesium, and calcium curves over the previous sampling date. It will be recalled that the  $\text{CaCO}_3$  treated pots produced the maximum crops. In the fallowed soil (Table IX), the carbonate treatment produced by far the largest amount of soluble material, as shown by the specific resistances. In this case, also, the greatest con-

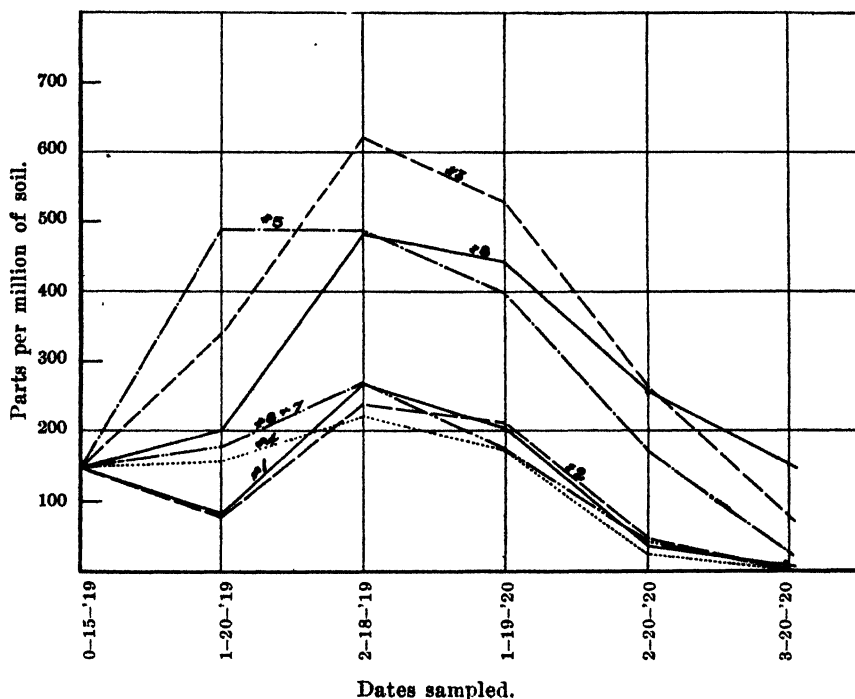


Fig. 19.—Effects of different treatments on nitrate-ion solubility.

centration of solutes appeared some weeks later than in the cropped soil. Magnesium was an exception. Here progressive solubility was gradual throughout. In both cropped and fallowed soils, gypsum and calcium carbonate were about equally effective in increasing magnesium solubility. A simple interchange of bases may possibly account for this. The solubility of the soil potassium is affected but slightly by the  $\text{CaCO}_3$  additions.

Figure 8 shows the effect of superphosphate treatment. Figure 4, which records similar data for the field plots, may also here be of comparative interest. A notable similarity is shown between the two. A comparison of figure 8 with the check pots, figure 5, shows the addition

of superphosphate to have practically doubled the amounts of water-soluble phosphorus, calcium, and magnesium throughout the duration of the experiment, while nitrate formation, contrary to expectation, was slightly depressed by it. The same holds true, in the case of nitrates, for the fallowed soils, although nitrification in both cases increased at first more rapidly in the presence of the soluble phosphorus. In this series the cropped and uncropped soils behaved very similarly as regards progressive solubility; the soil, when receiving an application of acid phosphate, apparently being able to maintain the important solutes at fairly high concentrations during crop withdrawals.

The results for the  $\text{NaNO}_3$  treatment are shown in figure 9. With the exception of the large amount of added nitrate, there is little difference between these soils and the checks. Thus, the nitrate application has had very little effect in increasing the solubility of this soil's constituents. This is in accordance with the recent work of Bauer,<sup>3</sup> who found that the presence of  $\text{NaNO}_3$  had no effect upon the availability of soil phosphorus, and of Jensen,<sup>23</sup> who concluded that nitrate applications had no effect upon potassium availability and actually decreased a soils soluble phosphate content. Spurway,<sup>43</sup> on the other hand, shows additions of  $\text{NaNO}_3$  to considerably increase the solubility of phosphorus and magnesium in the sandy soils which he investigated. The increases, however, are irregular, and the conditions imposed are most artificial. The fallowed soils receiving  $\text{NaNO}_3$  gave increases over the checks, although the increments were small in comparison with those noted for other treatments. The crop results further showed that nitrate applications were unwarranted. The specific resistances were here at first slightly lower than were those of the checks, due to the soluble nitrate application, but even this difference disappeared as the end of the growing period was reached.

The potassium sulfate application, although slightly increasing water-extractable soil materials, also had no enhancing effect upon yields. It was applied at the rate of 500 pounds per acre. Figure 10 shows that water-soluble lime and potash have each been slightly increased during the period of active growth. The solubility of the phosphorus has been unaffected, as has nitrate production. Magnesium has been increased. The results secured with potassium sulfate in the fallowed soil are in fair agreement with these. The general relationships hold, although slightly larger quantities of solutes appear in all cases. A slow progressive solubility is recorded for each

ion determined except calcium, which apparently assumes its maximum concentration about a month or six weeks after the addition of the potassium sulfate and thereafter gradually declines. None of the solubility increases is marked.

In treatment 7, superphosphate (one ton per acre), together with potassium sulfate (500 pounds per acre), was added. The effects upon the solubility of the various ions determined are shown in figure 11. As would be expected, the soluble salt content has been considerably increased. Nitrates, however, remain approximately as in the check pots. No uncropped soils carrying two-salt treatments were maintained. The yields here were a surprise—much below those where superphosphate alone had been used. This may be due to “alkali,” for analysis showed that  $\text{Na}_2\text{SO}_4$  was present slightly in excess of 0.2 per cent. Improper balance of salts may also be advanced as a possible explanation for the lowered yields, as sulfate, calcium, magnesium, and potassium-ions are present in large quantities, while nitrates are present in relatively low amounts.

In the last series, applications of superphosphate and  $\text{CaCO}_3$  were the treatments employed. The concentrations of the soil extracts were decidedly increased (see fig. 12), as shown by the conductivity measurements. The  $\text{Ca}$ -,  $\text{NO}_3$ -, and  $\text{PO}_4$ -ions especially showed greatly increased solubility. No tendency toward a decline in concentration during the period of rapid growth was evident. That soluble phosphate applications are superfluous when this soil is neutralized with lime is shown in figure 7. The ability of  $\text{CaCO}_3$  to set free soluble phosphorus from soil minerals has also been recorded by Fraps,<sup>11</sup> Hartwell and Kellogg,<sup>16</sup> Guthrie and Cohen,<sup>14</sup> and others.

In order to compare more easily the effects of the individual treatments upon the solubility of each ion, a second series of curves was prepared. The complete hydrogen-ion and conductivity data are also presented. Let us glance at figure 13, which shows the effects of each treatment (1 to 8) upon the periodically determined specific resistances. The determination of specific resistance upon soil extracts is at the present time meeting with considerable favor among soil investigators. In alkali studies, where large numbers of soils must be examined for total soluble salts, its use is certainly to be recommended. That considerable precision may be claimed for it has been shown by von Horoath,<sup>22</sup> who has proposed a soil classification based upon electrical conductivity. In figure 13, the high concentrations of treatments 8 and 3 (where  $\text{CaCO}_3$  was added) over the entire growth period are

well shown, while the low concentrations of the checks (1), the  $K_2SO_4$  pots (6), and the nitrate treated soils (5), are likewise emphasized. Numbers, 2, 4, and 7 occupy intermediate positions. That the yields may be closely correlated with soluble salt concentrations (conductivities) has been previously noted. A comparison with figure 20 emphasizes this fact.

Considerable interest attends the data presented in figure 14. Hydrogen-ion determinations were made periodically upon these pot soils throughout the experiment, much care being taken to secure accurate, comparative results. It was desired to ascertain whether or not, during the growth of the crop, any of the fertilizer treatments, except, of course,  $CaCO_3$ , had in any way altered soil reaction, and also whether or not, after adding  $CaCO_3$  to neutrality, any acidity subsequently developed. The abscissa shows the dates of sampling, while the ordinate is divided into the customary  $P_H$  units. The small, ten-gram samples used in making these determinations were carefully taken from the large monthly composite samples and were representative. The determinations were made upon the moist soils as soon as received from the greenhouse. A study of figure 14 shows that in treatments 3 and 8, sufficient  $CaCO_3$  had been added to maintain an alkaline reaction (above  $P_H7$ ), although the tendency to gradually decrease in alkalinity is shown at the last two sampling dates. While exactly the same amounts of  $CaCO_3$  were supplied in both cases, it will be seen that the addition of superphosphate in treatment 8 rendered this soil *more alkaline* at all times. The same tendency to induce alkalinity is shown where superphosphate is added alone (compare treatment 3 with treatment 1), the checks being the most acid soils of all. These results are in direct agreement with those of Conner,<sup>8</sup> who has shown that soils that had been treated with acid phosphate for twenty years were *less* acid than the untreated soils. Morse<sup>38</sup> has determined hydrogen-ion concentrations colorimetrically on certain plot soils. In agreement with other investigators, he claims that acid phosphate, even though used over a long period of years, produced no noticeable effect on soil reaction, while, where lime was occasionally used with it, at the rate of one ton per acre, the superphosphate apparently further enhanced alkalinity. Small, definite differences also existed between the checks and the soils receiving the neutral salt treatments. It will be seen that the  $K_2SO_4$  application has decreased the hydrogen-ion concentration throughout by at least three-tenths of a  $P_H$ —an amount too great to be considered experi-



mental error. This basic tendency of the other salts, while less pronounced, is, however, uniform and definite.

A decided upward trend of all of the curves (except 3 and 8) is noticeable from February 20 to March 20. The decreases in hydrogen-ion concentration are here too marked to be ascribed to error. A possible explanation for this is as follows: At the end of the growing season, a small fraction only of the nitrate still remains in these very acid soils (see figure 19). The soil solution must be practically saturated with  $\text{CO}_2$  due to rapid root growth and high organic matter content. When the large amounts of nitrate are absorbed and removed from solution, the bases formerly associated with this strongly acid radical may combine with the weak  $\text{H}_2\text{CO}_3$  forming bicarbonates of the strong bases (K, Na, Ca). Subsequent hydrolysis tends slightly to increase OH-ion concentration.

Another point which the writer deems of importance in connection with the reaction studies recorded is that strong soil acidity, per se, is *not necessarily* harmful to growth, and that it has in the past been over-emphasized as a cause of low productivity, especially in the case of leguminous crops. A glance at figure 21, together with the high yields secured in *all* cases, checks included, suffices to show that, even where such a "lime loving" legume as the pea is grown, other conditions being optimum, good results may be expected in the presence of high soil acidity. So far as the writer is aware there is no definite evidence in the literature to show that soil acidity of *itself* is the direct cause of infertility. Recent work at the University of California might be cited to show that heavy yields are often secured in solution cultures where hydrogen-ion concentrations are abnormally high. It is thus questionable whether *complete* neutralization, especially where high lime applications are necessary, is ever justified. Many cases have been noted where the satisfaction of a small fraction of the so-called "lime requirement" has increased yields to the same extent as have larger lime treatments.

The comparative calcium-ion concentrations in the variously treated soils appear in figure 15. In treatment 8, receiving both  $\text{CaCO}_3$  and superphosphate, we find the most soluble calcium. This is followed by  $\text{CaCO}_3$ , gypsum, superphosphate plus  $\text{K}_2\text{SO}_4$ , and superphosphate alone. The  $\text{K}_2\text{SO}_4$  and the  $\text{NaNO}_3$  treatments had little effect in setting free soil calcium.

The behavior of magnesium-ion is of interest in that it follows closely that of calcium-ion solubility. A comparison of figure 16 with

figure 15 shows that there are no exceptions to this statement. As magnesium was in no case applied to the soils\* in soluble form, there must have been a direct exchange of bases between this ion and those supplied in the treatments.

The solubility of soil potassium has been fully discussed. A direct graphical comparison of the treatments is shown in figure 17. The check soils (number 1) are the lowest in available potassium throughout, while, aside from the direct  $K_2SO_4$  treatments, gypsum is, in the soil under study, apparently superior to all others in setting free potash. The efficacy of the superphosphate additions is here doubtless due to this ingredient. Recent work of McCool and Millar<sup>22</sup> bears out this statement. Calcium carbonate is much less effective. Slight increases only result from the  $NaNO_3$  applications.

The percentage of water-soluble phosphate is unusually low in this soil and none of the treatments, except those directly supplying phosphate-ion, greatly alters its availability save  $CaCO_3$ , which has a slightly enhancing tendency toward the end of the experimental period. The check soils are a little below the others in the amounts of soluble phosphorus they contain, as shown in figure 18, although the differences are slight. That low concentrations of  $PO_4$ -ion are the rule in water extracts of soils has often been recorded. Certain data recently secured by the writer have shown that the same holds for the true soil solution as obtained by a direct pressure method. One to three p. p. m. of soil solution are here usually found. Work in this connection has been reported elsewhere.†

The nitrate-ion concentrations as plotted in figure 19 are of interest in that they closely agree with nitrification studies (not here reported). Except number 5, where  $NaNO_3$  was directly supplied, the  $CaCO_3$  treatments *alone* gave noteworthy increases. In all cases, however, sufficient nitrification may have taken place within this acid soil to supply crop requirements, although it should be recalled that a leguminous crop was grown.

While the results of these solubility studies apply to this soil alone, we are probably safe in considering them generally applicable to transported, low-lying acid clays and clay loams, comparatively high in organic matter and rich in nitrogen.

\* The superphosphate alone gave slight traces.

† See Soil Science, vol. 13.

## SUMMARY

The work herein reported embraces an investigation of an acid, marsh soil, unproductive for peas, by the use of certain of the more modern procedures. Both field and greenhouse experiments were conducted, a variety of fertilizing materials were employed, and soil-water-extracts, periodically made, were studied to ascertain the rates of formation, as well as the absolute amounts, of soluble salts formed in the soil when influenced by the different factors involved. This work has been supplemented by hydrogen-ion determinations and conductivity measurements. A detailed discussion of the results secured has been given in the body of the text, although a critical study of the data presented offers several points of theoretical interest.

Doubtless, the most important point made, aside possibly from the effects of the various treatments upon yields, is the remarkable *indirect* fertilizing action of certain of the chemical compounds when applied to this cropped, clay-loam soil. That this has been brought about by a process of ionic substitution, element for element, within the hydrated silicate molecules, thereby greatly increasing mineral solubility, is a probable explanation. Why certain bases, as calcium for instance, should be more active than sodium or potassium or why the  $\text{SO}_4$ -ion should be more reactive than either  $\text{NO}_3$ -ion or  $\text{PO}_4$ -ion are questions offering a good field for hypothesis and experiment.

In comparing field and greenhouse yields we see that while  $\text{CaCO}_3$  had no effect whatever in the field, in the pot experiment it gave the largest crop. With superphosphate the results were reversed. As this was an unusually dry year in the field, while in the greenhouse moisture conditions were maintained at optimum, an explanation may possibly lie in the comparative solubilities of these two compounds. The action of the  $\text{CaCO}_3$ , being in large part due to its indirect effect through enhanced nitrification, requires considerable quantities of water, while, on the other hand, if sufficient moisture is present to dissolve but a small portion of the superphosphate, enhanced yields should result in a soil deficient in available phosphorus. Another effect of the field application of acid phosphate was to increase permanently the solubilities of all of the soil constituents except  $\text{PO}_4$ -ion. Soluble phosphorus was directly supplied, yet at the end of two months no indications of such applications were apparent in the water

extracts. Similar conditions were observed in the greenhouse pot soils. It has been noted that small quantities (12 to 14 p. p. m.) of soluble aluminum were consistently found in this soil. A simple explanation of rapid phosphate reversion may thus be found in a direct union between superphosphate and soluble aluminum, with the formation of insoluble aluminum phosphate. In a soil rendered alkaline with lime, however, no such reaction could occur due to the precipitation of all soluble aluminum, either as the hydroxide or as

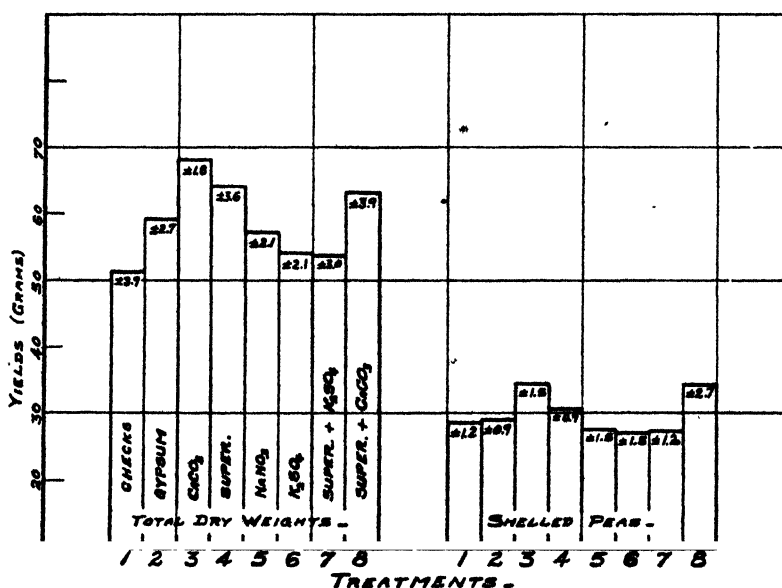


Fig. 20.—Comparative yields per pot of total dry matter and cured peas.

calcium aluminate,<sup>36</sup> as well as to the early formation of the reverted calcium phosphate which, so far as crops are concerned, is largely available. Such conditions do obtain in the  $\text{CaCO}_3$ -treated pots where maximum yields were registered and where moisture conditions were optimum. The curves here also show a slightly *enhanced* phosphate solubility which is maintained throughout the growing period.

A careful study of figures 5, 7, 11, and 12, together with comparative yields for these treatments (figure 20), casts some doubt as regards the power of soluble phosphorus to increase yields greatly in this soil unless soluble calcium also is present in adequate amounts. In figure 11 (superphosphate +  $\text{K}_2\text{SO}_4$ ) fairly large quantities of soluble  $\text{PO}_4$ -ion obtain (in fact, larger than appear in the  $\text{CaCO}_3$ -

treated pots), yet the yields are greatly in favor of the  $\text{CaCO}_3$  additions. Large percentages of soluble calcium are shown at all times in figure 7. In figure 11, however, less than one-half of these amounts is present, while magnesium-ion concentration in this case is almost equal to that of calcium-ion. These results may show that a certain balance of ions within soil solutions is essential for optimum plant growth.

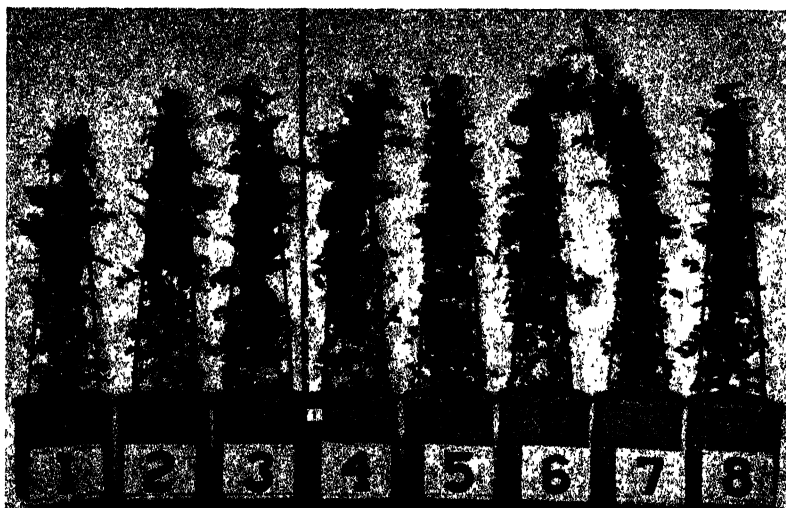


Fig. 21.—Plants one month before harvesting, showing the eight treatments.

Similar indications of the necessity for proper ionic ratios are shown in figures 7 and 9 where nitrates, phosphates, potassium, and calcium may be compared. The proportion of nitrates is high in both cases; the amounts of phosphates differ but 1 to 2 p. p. m., as is also the case with potassium, but calcium-ion is increased sixfold in the  $\text{CaCO}_3$  treatment where the maximum yields are recorded. Other examples might be given which indicate that where anions are high, cations must also be present in certain definite optimum proportions.

A glance at the periodical conductivity measurements on the extracts from the variously treated soils shows that they arrange themselves exactly in the order of productivity. This method has been shown to be of great value in the study of alkali soils where large quantities of soluble salts prevail. May it not be of still greater value, in the absence of alkali, where estimates of comparative fertility are desired?

Soil acidity has been fully discussed in the light of data here presented and, except in the presence of unusually high hydrogen-ion concentrations (below  $P_H$  4.5), it seems doubtful to the writer that acidity, per se, is ever the direct cause of low productivity provided sufficient concentrations of the basic ions (Ca, Mg, K) are present within the soil solution.

## CONCLUSIONS

The following general conclusions may be drawn as the result of these investigations:

These studies were carried out on an acid, drained, heavy clay-loam, marsh soil of the San Francisco Bay region which was unproductive for certain crops and carried small percentages of the white alkali salts, notably sulfates.

Nitrification studies showed that the addition of calcium carbonate to neutrality greatly increased nitrate production, while soluble phosphorus and potassium compounds, without lime, produced no effect. Ammonification was largely due to soil fungi, and the *Azotobacter* species were absent.

A statistical study of the factor of variability, where certain water-soluble ions within soil extracts were taken as the criteria, showed that apparently uniform field soils may vary greatly within small areas; this is in accordance with the recent work of Waynick and Sharp.<sup>48</sup>

In the field, water was apparently the limiting factor in crop production at the Marin Meadows Ranch during the 1919-1920 season. Under those conditions superphosphate applied at the rate of one ton per acre increased yields by approximately 25 per cent while liming to neutrality gave no increases over the check plots. The chemical control maintained throughout the duration of the field experiment showed that the acid-phosphate applications had greatly enhanced the solubility of soil K, Mg, and Ca, while nitrate production was affected but slightly. The rapid revision of soluble phosphate within this soil was thought to be due largely to the formation of aluminum phosphate, for a small amount of aluminum-ion was always present in water extracts of this soil. Ferrous compounds or other toxic materials aside from the white alkali salts were not found.

In the greenhouse, where moisture and temperature conditions were optimum, much larger plants were produced. A 35% increase

(over the checks) in yield of total dry matter attended the use of  $\text{CaCO}_3$ , when added to neutrality, and a 28% increase where superphosphate at the rate of one ton per acre was applied. The soils receiving gypsum treatments and the checks were about equal in productivity, while  $\text{NaNO}_3$ , and  $\text{K}_2\text{SO}_4$ , each supplied at the rate of 500 pounds per acre, gave slight but insignificant losses. The yields of dried peas followed in a similar order.

Nodule formation as affected by these treatments within this very acid soil is discussed. Nitrates completely inhibited it, while  $\text{CaCO}_3$  added to neutrality acted similarly (due doubtless to greatly enhanced nitrification). The application of soluble phosphorus increased nodule formation while potassium sulfate and gypsum produced no noticeable effects.

All of the chemical compounds added increased the concentration of the soil solutions under the growing crops when compared with the untreated checks, although marked differences between the several treatments were noted. A direct relationship existed between the concentration of solutes present in the soil extracts, as shown by conductivity measurements, and crop production. Gypsum was the most active liberator of the soil potassium and was equal to any other compound in effecting the solution of soil magnesium, while its action upon phosphorus availability and upon nitrate formation was nil. Calcium carbonate, when added to neutrality, was apparently the most effective soil solvent supplied, although its action was probably largely indirect. It occupies first place in effecting the solution of all ions, except potassium. In comparison with the checks, specific resistance was here decreased by almost one-half. This is doubtless due to the intensive nitrification which this treatment engenders. Nitrate production (from soil N) was nearly trebled, as was water soluble magnesium. Soluble calcium was increased many fold, and soluble K and  $\text{PO}_4$  were each increased by at least one-third. With the possible exception of nitrate-ion concentration, which likewise fell off in the fallowed soil, there was no declining tendency noticed on the part of any of the nutritive ions during maximum withdrawals by the heavy pea crop produced.

The enhanced solubility of soil minerals due to superphosphate applications is probably largely to be attributed to the gypsum which this material contains. Bearing in mind that approximately twice as much calcium was supplied in the gypsum treatments, the similarity between the two is strikingly shown in figures 6 and 8. Soluble phosphorus, of course, was directly supplied in the superphosphate.

Sodium nitrate had little effect upon this soil's solubility in water throughout the duration of the experiments here reported.

Potassium sulfate applications increased the amounts of Ca and Mg going into solution by possibly one-third, while nitrate formation and phosphate availability were apparently unaffected.

The results secured from the two-salt applications, both as regards yields and soil solubilities, were approximately the same as the average of the similar individual single-salt treatments.

A periodical study of hydrogen-ion concentration was carried out on each of the differently treated pot soils throughout the cropping period. All of the soils to which neutral salts had been applied were slightly but consistently less acid than were the checks, superphosphate especially tending to lower H-ion concentration. During heavy nitrate absorption there was a slow, definite increase in soil alkalinity. On the other hand, where calcium carbonate had been added to neutrality, a progressive increase in H-ion concentration was recorded. The question is discussed as to whether soil acidity, *per se*, is ever a direct cause of impaired productivity.

The results, when cropped and fallowed soils were compared, differed but slightly, the chief dissimilarity being that the water extracts of the fallowed soils reached maximum concentrations about a month later than did those of the cropped soils, and thereafter remained stationary or gradually decreased. Larger amounts of solutes were, as a rule, present in the uncropped soils but the same comparative relationships almost invariably held. A series of fallowed soils is therefore held to be here superfluous, little additional information being gained, while the labor involved is approximately doubled.

In conclusion, the writer wishes to express his indebtedness to Professor C. B. Lipman, under whose direction this work was done. Thanks for many helpful suggestions and criticisms are also due Professor D. R. Hoagland and Professor W. P. Kelley.



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THE EFFECT OF REACTION ON THE FIXATION  
OF NITROGEN BY AZOTOBACTER\*

BY

HARLAN W. JOHNSON AND CHARLES B. LIPMAN

## INTRODUCTORY

*Azotobacter* has been given considerable attention in the literature of soil bacteriology and it is thought that it plays an important part in keeping up the supply of nitrogen in many soils.

It has been recognized, almost since its first discovery, that *Azotobacter* is especially sensitive to the reaction of the medium in which it grows. The effect of acidity on the organism is so well established that its absence from a soil has frequently been taken as an indication that the soil needed lime. In fact, Christensen has worked out a method wherein he uses these organisms to determine the lime requirement of soils. The point has been repeatedly stressed that the acidity of soils must be neutralized if *Azotobacter* is to fix nitrogen effectively.

Since the influence of hydrogen-ion concentration on bacteria has been recognized, the following investigators have reported on its effect on these organisms.

Fred and Davenport<sup>1</sup> reported that *Azotobacter* is very sensitive, the limits for its growth being between  $P_H$  6.6 and  $P_H$  8.4 to 8.8.

Gainey<sup>2</sup> in a preliminary report states that in 90 soils studied all but 3 of the 37 in which no *Azotobacter* was found had a  $P_H$  value of 5.9 or less and all but 3 of those containing the organisms had  $P_H$  values of 6.0 or greater. In later papers<sup>3</sup> he reported that *Azotobacter* rapidly disappears when inoculated into soils whose  $P_H$  value is below 6.0 and that, in a study of 382 soils, using the hydrogen electrode for  $P_H$  measurements, 158 samples with  $P_H$  values below 6.0 and 20 above 6.0

\* This study was undertaken at the suggestion of Dr. C. B. Lipman, in whose laboratory the work was carried out.

contained no *Azotobacter*, while 165 samples whose  $P_H$  values were above 6.0 and 39 samples below 6.0 showed *Azotobacter*. The average nitrogen fixed in 186 samples containing *Azotobacter* was 7.9 mgs., while the average fixed in 181 samples lacking the organism was 4.6 mgs.

Waksman<sup>4</sup> in a study of cranberry soils found no *Azotobacter* in an unlimed soil with  $P_H$  values of 5.4 to 5.6, but in an adjacent limed soil with a  $P_H$  value of 6.2–6.4 found it to be present.

These investigators agree that slight acidity inhibits the growth of *Azotobacter*. None of them, however, reports on the effect of the reaction on the nitrogen fixing efficiency of the organism. Gainey reports the nitrogen fixed in the soils containing *Azotobacter* as compared with soils lacking them, but does not state the effect of the reaction in the soils where they were present.

The work here presented was undertaken to determine the effect of various hydrogen-ion concentrations on the ability of *Azotobacter chroococcum* to fix nitrogen.

#### EXPERIMENTAL

The organism used was a strain of *Azotobacter chroococcum* isolated from a California soil which in previous work had been found to be very efficient in nitrogen fixation and to produce abundant pigment.

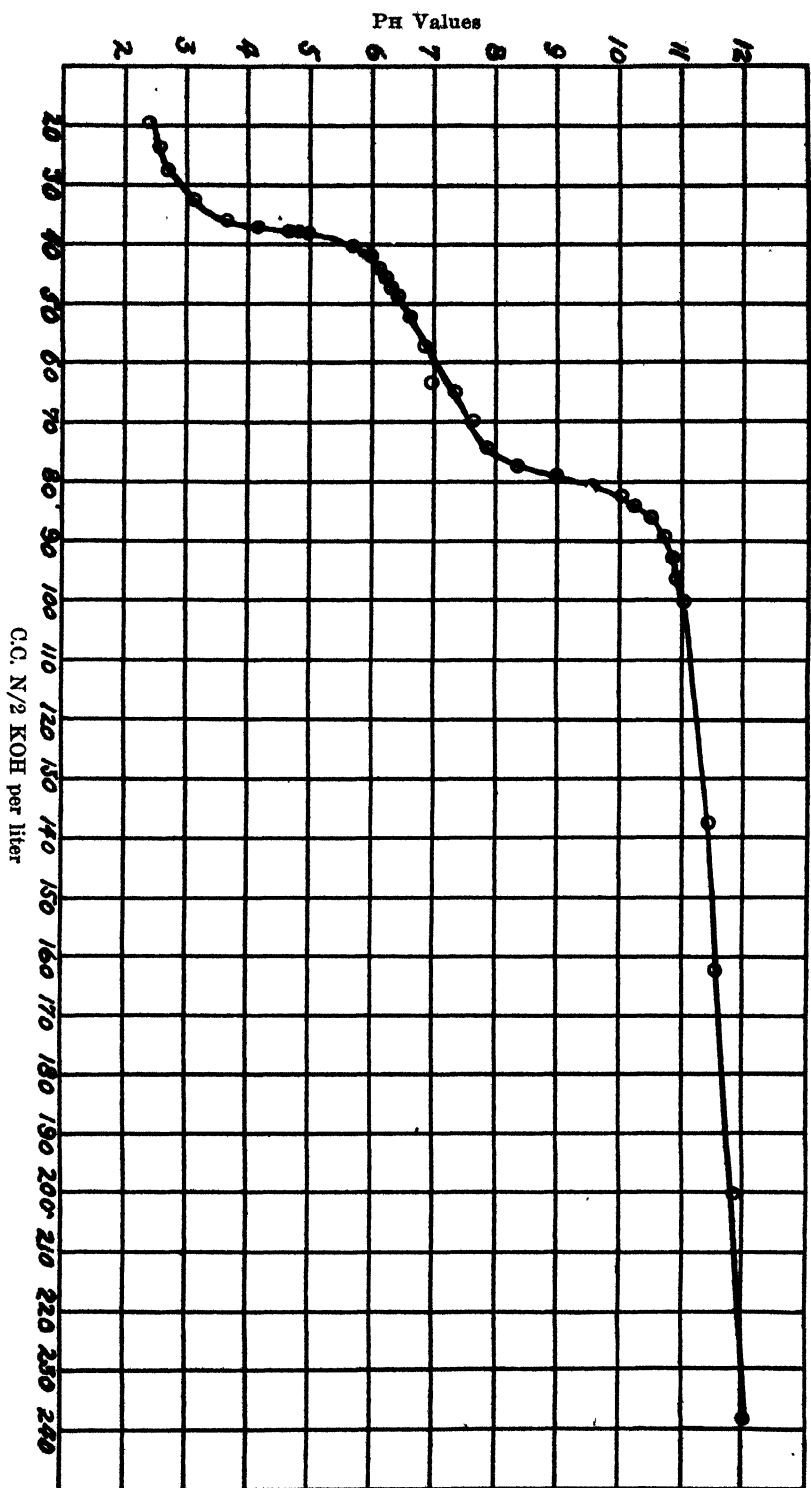
The medium was made up as follows:

Mannite,	15.0 gms.
MgSO <sub>4</sub> · 7H <sub>2</sub> O,	.2 gms.
NaCl,	.2 gms.
CaSO <sub>4</sub> · 2H <sub>2</sub> O,	.1 gms.
H <sub>3</sub> PO <sub>4</sub> ,	1.5 c.c.
Distilled water,	1000 c.c.

This solution was titrated with N/2 KOH to give definite  $P_H$  values, using the hydrogen electrode in the titration. The titration curve of the medium is shown in figure 1. The inoculations were made into 100 c.c. portions of the medium in 800 c.c. Erlenmeyer flasks. To secure uniform inoculation, 50 c.c. portions of the medium in a small Erlenmeyer flask were inoculated with *Azotobacter*, and after a characteristic membrane had formed the flask was shaken vigorously, and the suspension was used as the inoculum.



Fig. 1. Titration Curve of Medium Used



*The First Series*

In this series the medium was titrated to give  $P_H$  values of approximately 3, 4, 5, 6, 7, 8, 9, 10, and 11. These values changed during sterilization and incubation.

Four flasks of each reaction were inoculated and incubated three weeks at 28° C.

Three of the flasks of each reaction were left undisturbed. The others were used for  $P_H$  measurements, 5 c.c. being withdrawn at intervals from each for electrometric determinations.

The  $P_H$  values of the solutions in these flasks at the time of inoculation and at each of the succeeding periods are shown in figure 2.

After incubating twenty days the nitrogen in each of the undisturbed flasks was determined and the amounts fixed are given in table 1.

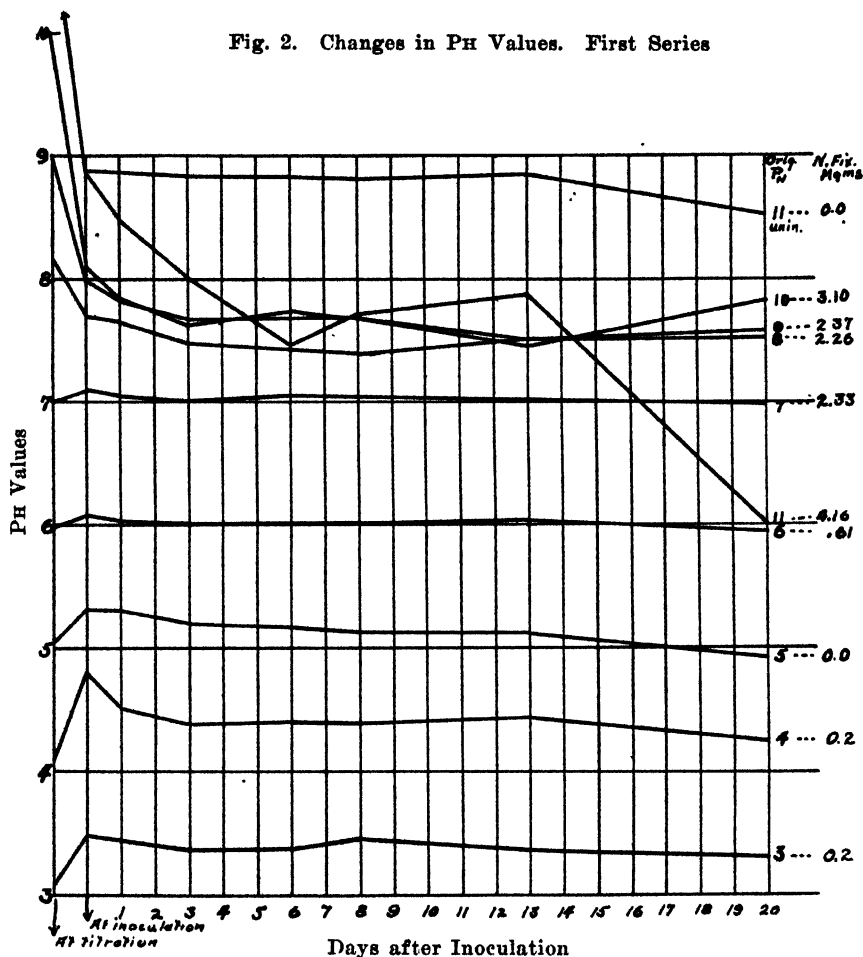
TABLE 1  
NITROGEN FIXED IN SOLUTIONS OF VARIOUS  $P_H$  VALUES  
Series 1

$P_H$ at time of titration	$P_H$ at time of inoculation	N Fixed Mgs.			Average
		Flask No. 1	Flask No. 2	Flask No. 3	
3.14	3.47	.07	.0	.0	.02
4.10	4.80	.0	.07	.0	.02
5.06	5.31	.0	.0	.0	.00
5.98	6.07	.70	1.12	.0	.61
7.00	7.08	1.82	2.66	2.52	2.33
8.15	7.69	2.59	1.75	2.45	2.26
9.01	7.98	2.42	2.28	2.42	2.37
10.04	8.08	2.40	3.38	3.52	3.10
11.03	8.86	4.95	4.11	3.41	4.16

It will be noticed that the  $P_H$  values of the media were not the same when inoculated, as at the time of titration. This may be due to incomplete reaction at the time of titration, although the titration required two days for completion. It might be due to the absorption of carbon dioxide by the more alkaline media, since these media showed the greatest changes and several days intervened between sterilization and inoculation. Probably both factors had their effect. It will be noted that the solutions with  $P_H$  values of 6.0 and 7.0, whose reactions remained remarkably constant, lie in the region which is shown by

the curve in figure 1 to be most highly buffered, while those with  $P_H$  values between 8.0 and 11.0, which are in a poorly buffered region, show the greatest changes. The growth of *Azotobacter* in the more alkaline solutions also affects the reaction. The more acid solutions, although in a poorly buffered region, show little change in reaction.

Fig. 2. Changes in  $P_H$  Values. First Series



The end point for nitrogen fixation on the acid side of the neutral point evidently lies very close to  $P_H$  6.0. At that reaction only a very small amount of nitrogen was fixed and in the solutions with lower  $P_H$  values no fixation occurred. The alkaline reaction inhibiting fixation was not reached in this series; in fact the largest amount of N was fixed in the most alkaline medium used.

### The Second Series

The second series was planned to find more accurately the lower critical  $P_H$  value and an alkaline reaction inhibiting fixation. Consequently solutions were made up with  $P_H$  values of approximately 5, 6, 6.2, 6.4, 6.6, 6.8, 7, 8, 9, 10, 11, and 12.

Four flasks of each  $P_H$  value were again inoculated and incubated, one of each  $P_H$  value being used as before for  $P_H$  determinations.

Only three  $P_H$  determinations were made, viz., at inoculation, ten days, and seventeen days later. It is regretted that no determination was made at the end of incubation. The reactions at the time of inoculation and later are shown in figure 3. Again those in the buffered region between  $P_H$  6.0 and 7.0 showed little change, while those above in the less buffered region were markedly changed. The change in the highly buffered solution of  $P_H$  12 is remarkable, but must most likely be due to absorption of carbon dioxide since sterilization did not materially change the reaction. It should be noted that an extra flask, uninoculated, changed exactly the same as the inoculated one.

These cultures were incubated thirty days, since the amounts of nitrogen fixed in the period of twenty days in the first series were rather small. The amounts of nitrogen fixed in this series are given in table 2.

TABLE 2  
NITROGEN FIXED IN SOLUTIONS OF VARIOUS  $P_H$  VALUES  
Series 2

$P_H$ at time of titration	$P_H$ at time of inoculation	N Fixed Mgs.			Average
		Flask No. 1	Flask No. 2	Flask No. 3	
5.00	5.34	.0	.0	.28	.09
5.99	5.97	.28	.84	1.96	1.02
6.20	6.29	5.88	4.76	4.20	4.95
6.41	6.49	3.36	4.62	5.18	4.38
6.63	6.70	3.64	4.90	4.34	4.28
6.83	6.83	3.78	4.90	4.34	4.34
6.97	6.91	7.50	8.12	2.66*	7.81
8.00	7.93	5.46	5.18	4.48	5.04
8.99	8.18	5.46	4.90	5.34	5.23
10.02	8.52	5.60	5.88	.42*	3.97
11.02	9.26	.14	8.26*	.0	.07
12.05	11.87	.0	.0	.14	.05

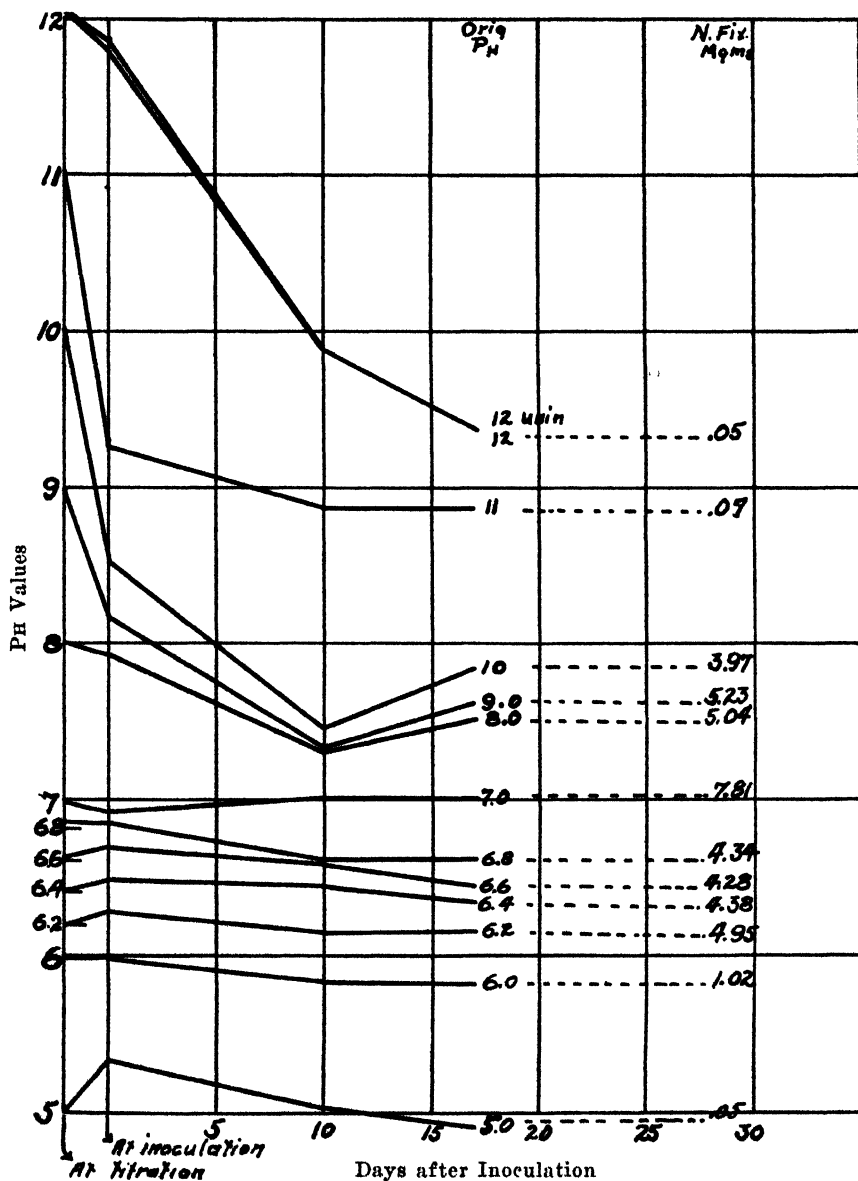
\* Not included in averages since very evidently some factor had affected the results.

The amounts of nitrogen fixed in this series were somewhat larger than those in the first series and show a somewhat different effect of reaction. In this series the neutral solution shows the greatest fixation.

It is evident again that  $P_H$  6.0 is near the critical limit for fixation. The very great increase in N fixed in the solution about .2  $P_H$  higher is remarkable. The lower limit for fixation is apparently very definite.

The upper limit for nitrogen fixation was reached in the solution whose  $P_H$  value at inoculation was 9.26.

Fig. 3. Changes in  $P_H$  Values. Second Series



## GENERAL DISCUSSION

These experiments were planned to determine the effects of hydrogen-ion concentration on nitrogen fixation by *Azotobacter*. The results obtained, however, can only be used to show that nitrogen fixation is not seriously affected until the critical limits of reaction are closely approached, when an abrupt decrease occurs in the amount of the nitrogen fixed. The variations in amounts of nitrogen fixed in solutions between these limits are not sufficient, considering the number of cultures used and the variations between cultures of the same reaction, to be of definite significance. The averages given in the tables are simply for convenience, and it is realized that a large number of flasks of each reaction would be necessary in order to secure conclusive evidence of the effects of various reactions.

The results show that there is an abrupt decrease in the amount of nitrogen fixed between  $P_H$  6.2 and  $P_H$  6.0, in other words, that the limiting hydrogen-ion concentration for good nitrogen fixation is a definite value between those two points. This corroborates the results of the previous investigators who found *Azotobacter* in soils whose  $P_H$  was 6.0 or above and none in soils with lower  $P_H$  values. It shows that we can expect no nitrogen fixation by *Azotobacter* in many of our soils, since it has repeatedly been shown that  $P_H$  values below 6.0 are frequently encountered. These organisms apparently react much more sensitively than do most of our other soil organisms, since ammonification, nitrification, and other forms of bacterial activity are active in soils whose acidity is higher than  $P_H$  6.

The alkaline limit for nitrogen fixation is apparently near  $P_H$  9.0, since in the first series the solution whose  $P_H$  value was 8.86 at inoculation showed good fixation, while in the second series the solution inoculated at  $P_H$  9.26 showed no nitrogen fixed. It is doubtful whether many soils ever attain such a reaction. Sharp and Hoagland report two soils whose alkalinity is greater, but these were exceptional alkali soils.

From this study, Fred's<sup>1</sup> limits of  $P_H$  6.6 and 8.4 to 8.8 would seem to be too narrow. Different strains of *Azotobacter*, however, may show variations in the effect of reactions on growth, and since this strain was an especially vigorous one its limits might be expected to be wider.

## SUMMARY

A vigorous strain of *Azotobacter chroococcum* was grown in solutions whose reactions were definitely determined by the hydrogen electrode. The nitrogen fixed in the solutions of each reaction was determined and the changes in reaction during incubation were measured.

It was found that the reaction of the solutions below  $P_H$  8.0 changed very little, those below  $P_H$  6.0 because no growth occurred, and those between 6.0 and 8.0 because the solution in this region was highly buffered.

Above  $P_H$  8.0 the reaction changed greatly, possibly due to incomplete reaction of the alkali at the time of titration, but more probably due to absorption of carbon dioxide by the strong alkali.

The amount of nitrogen fixed was not greatly affected by reactions between  $P_H$  values of 6.2 and 8.8 although reactions around  $P_H$  7.0 and 8.0 seemed to be most favorable. Slight changes outside of these values caused an abrupt decrease in fixation.

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THE TOXICITY OF COPPER SULFATE  
TO THE SPORES OF TILLETIA  
TRITICI (BJERK.) WINTER

BY  
FRED N. BRIGGS

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THE TOXICITY OF COPPER SULFATE  
TO THE SPORES OF  
TILLETIA TRITICI (BJERK.) WINTER\*

BY  
FRED N. BRIGGS†

INTRODUCTION

Copper sulfate has long been recognized as an effective fungicide for the control of *Tilletia tritici* (Bjerk.) Winter. The usual treatment is to dip seed wheat infected by the organism in a copper sulfate solution. That this treatment greatly diminishes the number of diseased plants is certain, but just how the individual spores are affected physiologically by the copper has never been fully determined.

REVIEW OF LITERATURE

According to Evans,<sup>4</sup> "Tessier, 1889, seems to have been the first to use copper compounds for the prevention of smut." In a work published in 1807, Prevost<sup>7</sup> gives a careful account of the effect of copper on the spores of wheat smut, *Tilletia tritici*. Stevens<sup>9</sup> studied the toxicity of a large number of chemical compounds and concluded that all the copper salts agree closely in their toxic action on fungous spores. Duggar<sup>3</sup> made an extensive study of spore germination as

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† Assistant Pathologist, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture (in coöperation with the California Agricultural Experiment Station). The writer wishes to express his indebtedness to the United States Department of Agriculture for the privilege of pursuing post-graduate studies; to Professor W. W. Mackie, of the University of California, under whom this work was done, for his kind suggestions and helpful criticisms; to Professor J. P. Bennett for the use of his laboratory and for his many kind suggestions.

affected by certain chemical, as well as physical, stimuli. He found that fungi were stimulated very little, if any, by copper sulfate and that they tolerate only very dilute concentrations of copper sulfate. Clark<sup>1, 2</sup> studied the toxicity of copper sulfate to 15 fungi which represented fairly well the natural groups, and found that 12 forms represented a range of lethal concentration of .0168 N to .0099 N, or slightly less than 70 per cent variation. He also found that copper sulfate was much more toxic when dissolved in pure water than when dissolved in any other medium. Hawkins,<sup>5</sup> using distilled water, found that a .00006 N concentration of copper nitrate practically inhibited the growth of *Glomerella cingulata*.

In summing up the work of previous investigators, it is readily seen that the growth of most fungi is inhibited by rather low concentrations of copper compounds.

## METHODS

In starting this research, the first problem was to determine the conditions under which maximum uniform germination could be secured.

Stakman<sup>8</sup> writes that rather uncertain and capricious germination was noted by Prevost, De Candolle, Tulsane, Kühn, Fischer von Waldheim, Brefeld, and others. He found that germination required from two to four days in water at room temperature and that all nutrients except soil infusion exerted a harmful effect on germination. McAlpine<sup>6</sup> also germinated the spores in water in two or three days. Wilcox<sup>10</sup> was not able to obtain more than 8 to 10 per cent germination in distilled water, and that only after a period of twenty-five to thirty days.

*Culture solutions.*—In preliminary experiments to determine the best medium for germination the following solutions were used:

- No. 1. Water extract from Yolo sandy loam soil.
- No. 2. Same as No. 1 diluted to one-half strength.
- No. 3. Same as No. 1 diluted to one-quarter strength.
- No. 4. Water extract of San Joaquin sandy loam soil.
- No. 5. Same as No. 4 diluted to one-half strength.
- No. 6. Same as No. 4 diluted to one-quarter strength.
- No. 7. Distilled water.
- No. 8. Tap water.

Extracts of soil were made by mixing one volume of soil with two volumes of water and autoclaving for one and one-half hours at 17 pounds pressure. The liquid was filtered off under pressure and sterilized.

Solution No. 5 gave the highest per cent and most uniform germination and was therefore used for the experiment.

Using Baker's analyzed copper sulfate, a .1N stock solution was made up. The concentrations required for this experiment were then made up by a series of dilutions and were prepared without the measurement of less than 10 c.c. in any case. Standard pipettes and volumetric flasks were used.

*Temperature.*—In preliminary experiments to determine the optimum temperature for germination, tests were made at room temperatures and controlled temperatures, 48° F., 53° F., 58° F., and 63° F. The latter temperatures were maintained by an automatically regulated chamber placed in a 40° F. cold storage room. Based on the results of the above experiments, it was decided to conduct one set of experiments at a controlled temperature of 58° F. and one set at room temperature which varied from 56° to 62° F.

*Method of culture.*—Two methods of culture were used: (a) the sealed hanging drop method as described by Clark<sup>1</sup> and Duggar<sup>2</sup>; and (b) the ventilated hanging drop. The latter was prepared by supporting the cover glass on two strips of paraffin 4 mm. square and 25 mm. long. By heating the slides the strips were sealed to them, and the cover glasses were made fast by pressing the edge with a hot needle. These cultures were kept in a moist chamber, in order to keep the drop from evaporating.

*Examination of cultures.*—Cultures were examined on the fourth day, and each day thereafter until they were nine or ten days old. Counts of ten spores were made from each of five different fields and the average per cent of germination recorded.

## DATA AND DISCUSSION

The data showed that there was little, if any, difference between the germination at the laboratory temperature and that at the controlled temperature. Likewise the germination in the sealed hanging-drop cultures was not essentially different from that in the ventilated hanging-drop cultures.

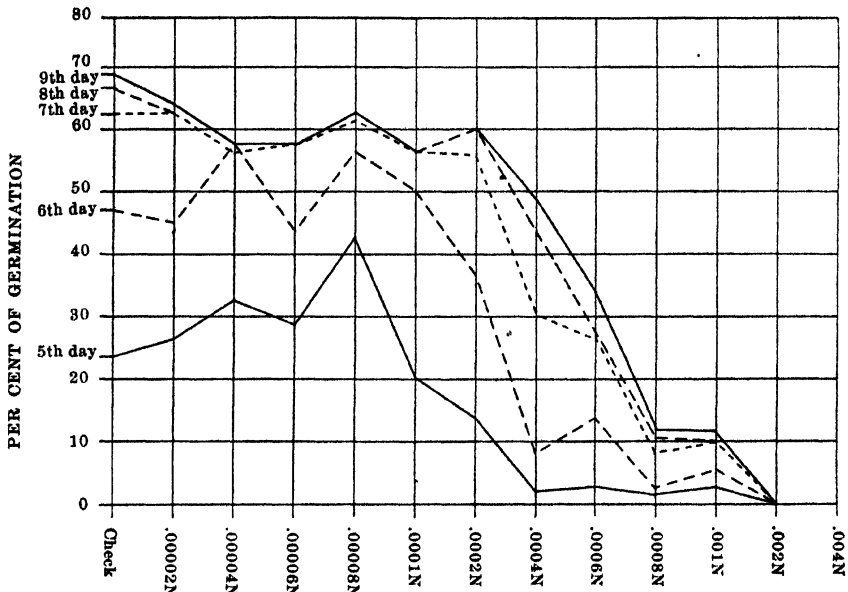


Fig. 1. Concentration of copper sulfate.

Under all conditions germination was somewhat erratic. Frequently duplicate mounts showed variations of 30 to 50 per cent in germination, but this was probably due, to some extent, to contamination by moulds, which frequently occurred toward the end of the incubation period. The technic followed in making up the mounts undoubtedly was responsible for some of the erratic germination. A small drop spread in a thin layer gave better germination than a large drop. The number of spores in proportion to the amount of solution was probably another factor. In a given drop the amount of copper for each spore would decrease as the number of spores increased. So, with a few spores, there might be a decided decrease in germination, while with a large number of spores there would be little decrease.

Since there was so little difference in germination under the different conditions of this experiment, the results were averaged together and are presented in a graph (fig. 1).

It will be noted from the data represented in the graph that an .002N solution of copper sulfate was sufficient to inhibit the growth of the spores of *Tilletia tritici* under the condition of this experiment. Cultures of .002N to .008N were kept for 20 days without any germination. A soil extract of a different concentration probably would have given a different point of inhibition. Clark<sup>2</sup> found the lethal concentration of copper sulfate to be .0076N when a beet decoction of normal strength was used; while it was .0034N, or approximately one-half when the decoction was diluted to four volumes.

Concentrations of .0008N and .001N caused a decided decrease in percentage of germination. The promycelia were very short and distorted, in many cases never reaching a length greater than 20 to 30 $\mu$ . No sporidia were found in cultures of this concentration, and because of their weakened condition it is very doubtful if any of these spores would be capable of infecting a wheat plant.

The concentration of .0006N copper sulfate caused many signs of abnormal germination, but frequently a spore would germinate in a perfectly normal manner, in so far as one could determine from a superficial examination. In the more dilute copper solutions, germination apparently was normal with no depression of any very great consequence in the percentage of germination, while in the more concentrated copper solutions there was some delay in germination and a very marked decrease until the point of inhibition was reached.

## CONCLUSIONS

Under the conditions described the following conclusions may be drawn:

1. In a culture solution consisting of a water extract of San Joaquin sandy loam soil, a .002N concentration of copper sulfate is sufficient to inhibit the germination of spores of *Tilletia tritici*.
2. In concentrations of .0008N and .001N there was very little germination and that which occurred was decidedly abnormal in character, the promycelium being very short and distorted.
3. It is doubtful if the abnormal promycelium obtained in concentrations of .0008N and .001N was capable of causing any infection.

4. Germination of spores in a .0006N copper sulfate solution was abnormal except for occasional ones which seemed to develop in the normal way.

5. In the more dilute copper sulfate solution, .00002N to .0004N, germination apparently was normal. Occasional spores in concentrations of .0004N and .0006N showed some copper injury.

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**INFLUENCE OF REACTION ON INTER-RELATIONS**  
**BETWEEN THE PLANT AND ITS**  
**CULTURE MEDIUM**

BY

J. J. THERON

**UNIVERSITY OF CALIFOR**  
**BERKELEY, CALIFORNIA**  
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# INFLUENCE OF REACTION ON INTER-RELATIONS BETWEEN THE PLANT AND ITS CULTURE MEDIUM

BY

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(Contribution from the Laboratory of Plant Nutrition, College of Agriculture)

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## I. INFLUENCE OF THE REACTION OF THE MEDIUM UPON THE PLANT

### INTRODUCTION\*

The reaction of the substrate in which the roots of plants develop is of obvious importance to the life of the plants. Earlier plant physiologists have neglected this factor, and it was not until recently as a result of studies on the intensity of the acidity of the soil solution of certain soils, on the one hand, and the relative resistance of different varieties of plants to alkaline conditions in certain types of 'alkali soils,' on the other, that the significance of this factor was fully realized.

Since the preliminary studies of Pantanelli<sup>26</sup> and Hoagland,<sup>15</sup> several investigators have attacked the problem. The practical, as well as theoretical importance of a more thorough understanding of the influence of the reaction of the culture medium on the growth and metabolism of plants seemed to warrant the investigation here described.

The object was twofold: (1) a study of the effect of various concentrations of hydrogen ions on the external appearance and growth of the more common agricultural plants; (2) the effect of the reaction on the metabolism of these plants.

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\* The writer wishes to acknowledge his indebtedness to Professor D. B. Hoagland for advice and kindly suggestions during the course of the investigation.

For obvious reasons, it was impossible to employ more than a few types of plants to accomplish these aims; hence plants were selected which were adapted to the methods of experimentation, and which may be considered as representative of the majority of field crops. These were alfalfa (*Medicago sativa*), cotton (*Gossypium herbaceum*, Durango variety), cucumbers (*Cucumis sativa*, White Spine variety), Bermuda grass (*Cyanodon dactylon*), corn (*Zea mais*, White Dent field corn), barley (*Hordeum vulgare*, Beldi variety), and peas (*Pisum sativum*, Canada field), the latter two being the principal ones used in the study of the inter-relations between the metabolism of the plant and the reaction of the culture solution.

Owing to the complexity of the soil and the reactions taking place therein and because of the many complicating factors which enter when sand cultures are used, solution cultures were employed exclusively.

#### EXPERIMENTAL

Baker's analyzed salts and the ordinary distilled water of the laboratory were used in making all culture and stock solutions. The stock solutions were those used regularly in this laboratory. Table 1 gives the weights of salts added to 18 liters of water to make up those solutions.

TABLE 1  
WEIGHTS OF SALTS DISSOLVED IN 18 LITERS OF THE DISTILLED WATER TO  
MAKE UP THE STOCK SOLUTIONS

Solution I	Solution II	Solution III
KNO <sub>3</sub> : 1200 grams MgSO <sub>4</sub> : 679 grams	Ca(NO <sub>3</sub> ) <sub>2</sub> : 1805 grams	KH <sub>2</sub> PO <sub>4</sub> : 900 grams

In table 2 is given the composition of the culture solution used throughout in this investigation (except where otherwise stated). This solution was made by adding 80 c.c. of solution I, 40 c.c. of solution II, 480 c.c. of solution III, and 24 grams of NaNO<sub>3</sub> to 44 liters of water.

TABLE 2  
COMPOSITION OF CULTURE SOLUTION EXPRESSED AS EQUIVALENTS, PER LITER

K	NO <sub>3</sub>	H <sub>2</sub> PO <sub>4</sub>	Ca	Mg	SO <sub>4</sub>	Na	P <sub>H</sub>
.0052	.0087	.0040	.0011	.0007	.0007	.0064	4.9

Iron was supplied in the form of ferric tartrate, one cubic centimeter of a 0.5 per cent solution being used per liter of culture solution.

In figure 1, the titration curve of the solution is reproduced. This was obtained colorimetrically. By interpolation the amounts of acid or alkali to be added to eleven liters of the solution to obtain any desired  $P_H$  within the useful range can be found from this graph.

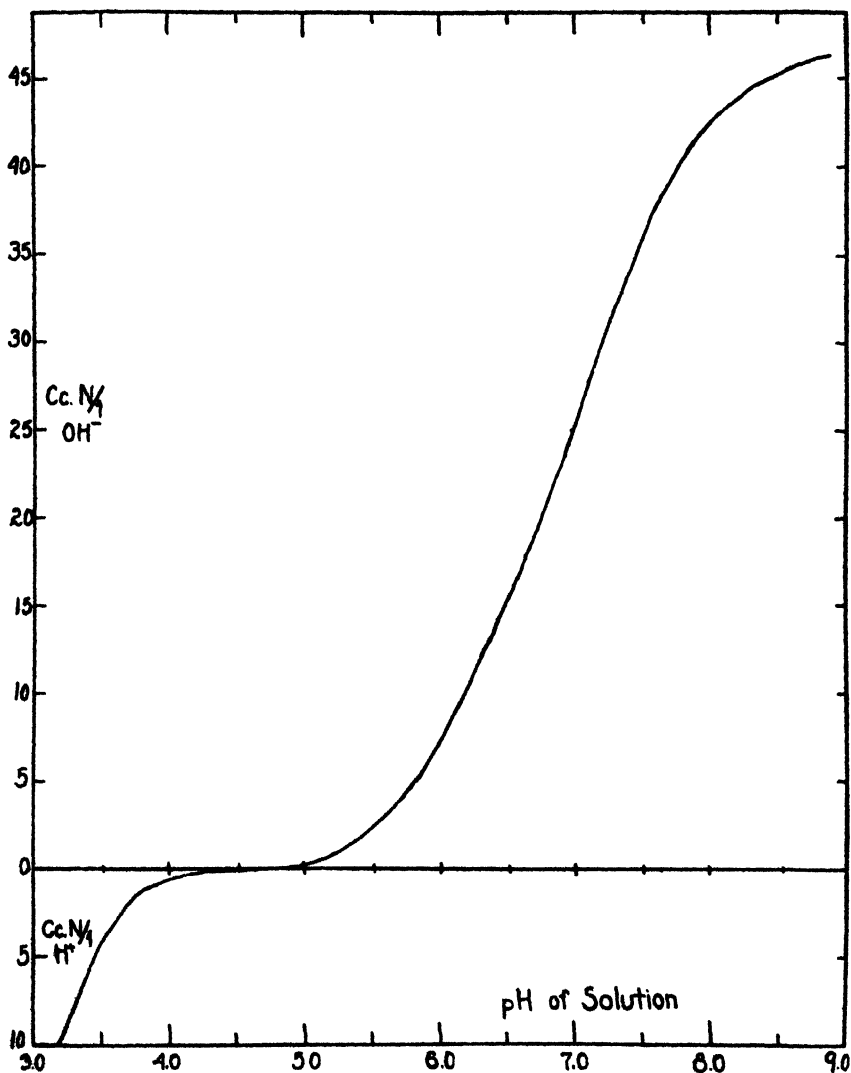


Fig. 1

In an effort to keep the composition of the solution as constant as possible over the entire range of reactions used, the concentrations of Ca and Mg were kept low, and were regulated by the amount of

calcium which will remain in solution at  $P_H$  8.0. A precipitate usually occurred at  $P_H$  8.5 and often at 8.0 after a few days. A comparatively high concentration of phosphate was used, on the other hand, in order to increase the buffer effect of the solution. Unfortunately, the buffer effect varies over different ranges of reactions.

This serious defect may be partly remedied by the addition of an acid with a dissociation constant of about 4.5 and a base with a constant of about 5.5. The only non-toxic acids having the desired constant are organic acids, e.g., citric acid. Owing to the danger of excessive bacterial growth in solutions containing organic matter, however, these non-toxic, organic acids cannot be used satisfactorily (Salter and McIlvaine<sup>27</sup>). Ammonium hydroxide may be used to supplement the buffer effect of the phosphate at  $P_H$  8 to  $P_H$  10, but the advantages to be gained here are small and the presence of the ammonium ion may introduce complicating factors.

Growth in the culture solution was very satisfactory if changes were made weekly. Sulfuric acid and sodium hydroxide were used to regulate the  $P_H$  values of the solutions. Measurements of the reaction were made by the indicator method of Clark and Lubs.<sup>18</sup> Frequent use was also made of a Hildebrand-type hydrogen electrode.

The plants were germinated between sheets of wet paper toweling and the usual methods of solution culture technique followed. At first properly covered Mason jars of 950 c.c. capacity were used as containers. In each jar, three plants were grown, ten jars being employed for each  $P_H$  tested.

The plants were grown in series of solutions having the following initial values: 4.0, 4.5, 5.0, 6.0, 7.0, 8.0, 8.5, and 9.0. Since the reaction changed very rapidly in the direction of neutrality, the solutions were renewed every second day. These frequent renewals, however, did not prevent the reactions of the solutions from being changed considerably during the later stages of growth. The maximum changes in reactions are tabulated in table 3. The plants were grown from 3 to 4 weeks, within which time sufficient growth was made to determine at which reactions they were affected adversely.

TABLE 3  
MAXIMUM AND MINIMUM VALUES OF THE REACTIONS AT TIME OF CHANGE

Initial $P_H$ of Series	4.0	4.5	5.0	6.0	7.0	8.0	8.5
Maximum and minimum $P_H$ at time of change	4.1-4.4	4.6-5.0	5.0-5.2	6.0-6.1	7.0-6.9	8.0-7.9	8.4-8.0

We assume naturally that the slightly increased concentrations of Na ions and  $\text{SO}_4$  ions used in regulating the  $\text{P}_\text{H}$  values of the different solutions have no effect on the plant, and that all differences in the external characteristics are caused directly or indirectly by the activities of the hydrogen or hydroxyl ions. The influence of the different reactions was determined by the relative weights of plants grown in the different solutions, the length and appearance of the roots, and the height and color of the tops.

The general effect of excessive acidity is very characteristic, and is the same for all the plants used in the experiment. If the culture solution is injuriously acid, the roots thicken and soon become a dull white in color which is easily distinguishable from the silky white appearance of normal roots. Depending upon the degree of acidity, the roots may stop growing in length entirely or may grow only slowly. In the latter case, they become knobby, because of the excessive development of laterals which penetrate the outer layers of the root with apparent difficulty. Lateral roots may develop to within a few millimeters from the growing tip. If the injury is not too severe the roots recover very rapidly when placed in a more favorable solution. The tops of the plants show a marked stimulation in growth and general vigor, as a rule, when compared with the plants grown in a more favorable solution. The stimulation, however, is of short duration and after two weeks they begin to lag behind. Similar results were obtained by Hixon.<sup>14</sup>

An injurious alkalinity of the culture solution is very readily recognized by a yellowish discoloration of the roots. In extreme cases, the roots become gelatinous and soon disintegrate. At first the tops showed no differences in size and vigor as a result of injury to the roots when compared with the tops of plants growing in a more favorable solution. After two to three weeks, however, a decided stunting was noticeable, and chlorosis of the new leaves set in.

Chlorosis is generally ascribed to the lack of available iron. This was probably the main cause of the chlorosis of those plants grown in the alkaline series. That excessive concentrations of hydroxyl ions, however, may cause chlorosis directly seems certain from the following considerations.

A distinct test for iron could be demonstrated in a solution kept at  $\text{P}_\text{H}$  8.5 even after chlorotic plants had been growing in it for a week. Cucumbers and alfalfa will show chlorosis at  $\text{P}_\text{H}$  7 within two weeks; at this reaction neither barley nor peas show any chlorosis

even after nearly two months' growth. By this time, one would expect the supply of iron stored in the seeds of the latter plants to be depleted. Gile and Carrero<sup>8</sup> found that ferric tartrate supplied the necessary iron to plants grown in solutions which they thought to be alkaline.

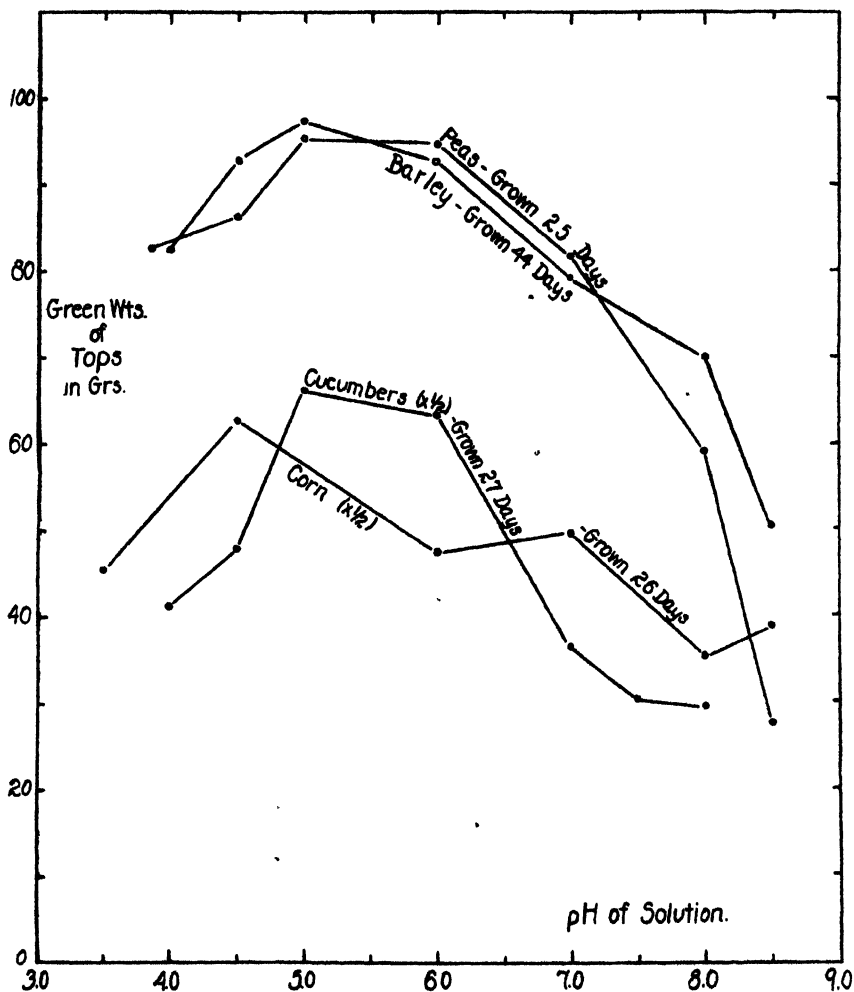


Fig. 2

It may be objected that the iron is not translocated from the roots to the tops in the case of the plants growing in an alkaline solution, and hence the plants are nevertheless suffering from a lack of iron. The reaction of the root juices, expressed after freezing, indicates,

however, that it is hardly possible for the increased concentration of the hydroxyl ions to interfere with the translocation.

All the plants grown on the acid side of  $P_H$  6.0 were deep green; above this reaction the color gradually became paler green, merging into complete chlorosis at the higher  $P_H$  values.

It is apparent that the plant is influenced strongly by the reserve store of food material in the seed. Great care must be taken in making any deductions from the experiments in which the plants have been grown for a short period of time only.

A much more thorough study of the problem has been made using the technique described below. The results of the experiments just discussed are therefore summarized in table 4 without further detail here.

TABLE 4  
EFFECT OF ACIDITY AND ALKALINITY ON GROWTH OF VARIOUS PLANTS

Plant	$P_H$ injuriously acid	$P_H$ at which optimum growth takes place	$P_H$ injuriously alkaline	Remarks
Alfalfa	4.2-4.5	4.8-6.0	7.0	Very sensitive
Cotton	4.2-4.5	5.0-7.0	8.0	Fairly resistant
Cucumbers	4.2-4.5	4.8-6.0	7.0	Very sensitive
Barley	4.2-4.5	4.5-7.0	8.0	Resistant
Bermuda grass	4.2	4.5-8.0	9.0	Highly resistant

All the varieties of plants tested, except the Bermuda grass, were affected adversely by approximately the same intensity of acidity. Alfalfa and cucumbers were affected much more severely, a fact which is correlated with their greater sensitiveness to alkaline conditions. In all cases, the best growth was made when the reaction of the culture solution was between  $P_H$  5 and  $P_H$  6. It may be of interest here to note that Fred and Davenport<sup>8</sup> found the critical point for the growth of alfalfa bacteria to be at  $P_H$  4.9. This reaction is well within any possible critical range for the host plant.

The use of the technique described above involves an excessive amount of labor and errors are unavoidable. At best, we are unable to control the reactions of the solutions satisfactorily. The advantages of the technique evolved later and described below will at once be evident.

Whereas with the former technique, 30 plants were grown in ten different jars at every  $P_H$  value in the experiment, all 30 plants were now grown in one three-gallon (eleven liter) earthenware crock. The



plants were supported as before in perforations on a cork sheet made by binding together three 12" x 4" x  $\frac{3}{8}$ " cork slabs with two strips of wood nailed on the edges. To prevent lateral movements, small pieces of wood were nailed on the underside of the overlapping corners. Slabs of wood  $\frac{7}{8}$ " thick serve the purpose even better, since the plants can be supported more firmly in them. These slabs must be soaked thoroughly in hot pure paraffin so as to prevent the absorption of water. By growing all thirty plants in this large volume of solution, the effect of the inherent variability of the plants is minimized and most of the experimental errors are eliminated. The roots can

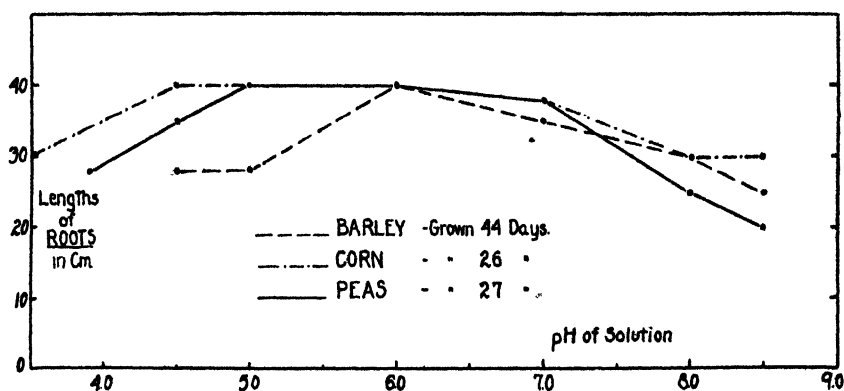


Fig. 3

be inspected readily and the  $P_H$  of the solution can be adjusted conveniently, rapidly, and as frequently as desired. The  $P_H$  is adjusted by withdrawing 5 c.c. samples of the solution and determining the reaction colorimetrically. The amounts of acid or alkali which must be added to bring the  $P_H$  to the original value are read off from figure 1, and the required quantities added to the solution. Figure 1 applies strictly to only the fresh solution. Within a week, however, the composition of the solution did not change sufficiently to invalidate the method. The solutions were changed every week and the  $P_H$  adjusted twice a day, i.e., in the morning and evening. During the later stages of growth, this becomes necessary more frequently in the case of plants growing at the reactions  $P_H$  4.0–5.0 and  $P_H$  8.0–9.0. Over these ranges, the buffer effect of the solution is relatively small and the power of the plant to change the  $P_H$  of the solution is increased (see figs. 4 and 5). All the plants experimented with showed a tendency to change the  $P_H$  of the solutions to a value between  $P_H$  6.2 and 6.8.

Because of the lack of time, it was not possible to subject all the plants used in the former experiments to these better controlled methods. This was done, however, with four widely different types of plants, namely, barley, peas, cucumbers, and corn.

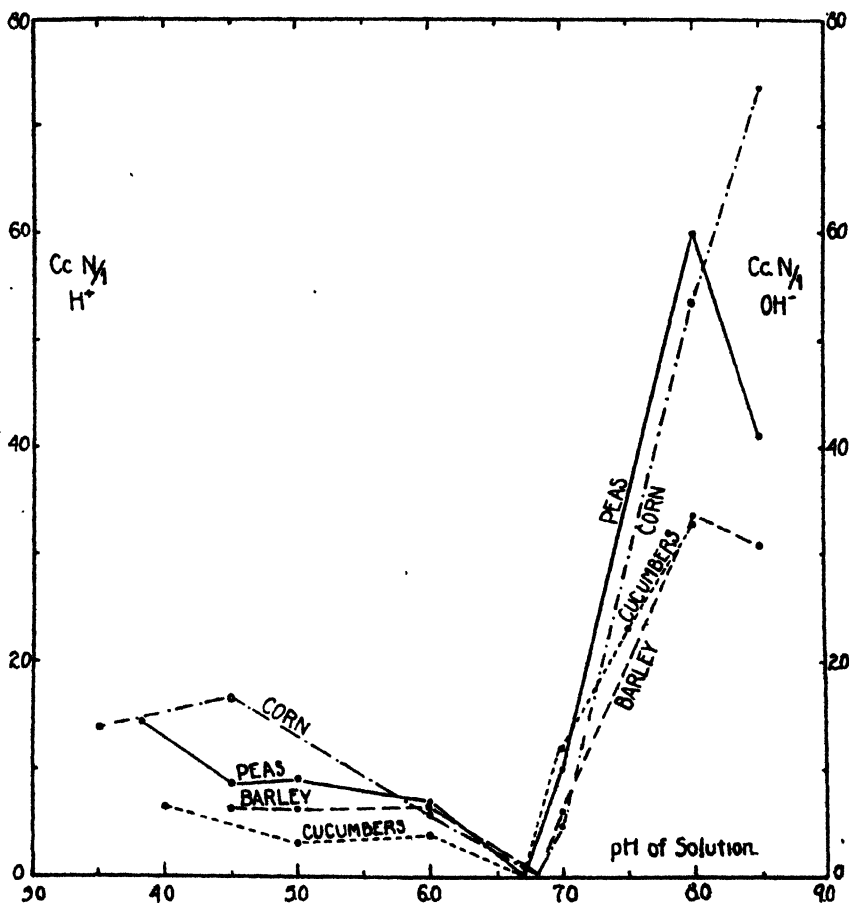


Fig. 4

In table 5, one experiment with peas is summarized. In the first column are given the desired reactions of the solution, in the second the highest and lowest  $P_H$  values reached during the course of the experiment, and in the seventh the number of cubic centimeters of normal hydrogen (sulphuric acid), or normal hydroxide ions (sodium hydroxide), added during the entire period of growth to replace that neutralized by the plants.

In figure 2, the green weights of the tops of 30 plants of the four types are plotted against the  $P_H$  of each series (see column 1, table 5), and in figure 3, the length of the roots. Since neither the change of  $P_H$  nor the change in the total molality of acid or base with time is an arithmetic function, it is impossible to calculate an average  $P_H$ . The true average  $P_H$  values differ only by a small amount from the desired  $P_H$  such that the given curves are not greatly different from the curves which would be obtained if the true average reactions were used. The differences are within the limits of the experimental error.

TABLE 5  
SUMMARY OF A TYPICAL EXPERIMENT WITH PEAS

Desired $P_H$ of series	Maxi- mum range of $P_H$	Days grown	No. of plants	Green weight of tops gms.	Length of roots cms.	C.C N/1 Reagent neutralized "		Remarks
						Acid	Alkali	
3.9	3.9-4.0	25	30	82.8	28	14.8		Roots severely injured
4.5	4.5-4.7	25	30	86.2	35	8.8		Very slight injury to roots
5.0	5.0-5.2	25	30	95.3	40	8.9		Best growth
6.0	6.0-6.1	25	30	94.2	40	7.0		Best growth
7.0	7.0-6.9	25	30	81.1	38		8.0	
8.0	8.0-7.9	25	30	59.4	25		60.0	Tops slightly chlorotic
8.5	8.5-8.3	25	30	27.6	20		41.0	Roots badly injured. Tops chlorotic

The juices of the plants were needed for other experiments, so the dry weights were not determined. For the present purpose, the green weights of the tops give a reliable criterion of the general vigor and size of the plants. The differences in the weights of the barley and pea plants can hardly be considered as significant in themselves on account of the inherent variability of the plants. If the observations on the other effects are taken into consideration, however, it becomes evident that the small differences in weight are true expressions of the effect of the corresponding reactions on the growth of the plants.

The maximum changes in  $P_H$  brought about by all four types were the same as that given for peas in column 2, table 5, except in the case of corn, grown in the alkaline solution, where the reaction frequently reached the  $P_H$  8.1. Since four widely different types of plants were used, the curves may be considered as a definite measure of the effect of the reaction of the culture medium on the growth of

most agricultural plants as indicated by the yield. They show unmistakably that the optimum range of the reaction for the propagation of these plants in solution cultures is between  $P_H$  4.5 and 6.0, and agree substantially with the results found with the earlier method of experimentation (see table 4). In figure 4, the amounts of normal acid or alkali neutralized by 30 plants during the first 25 days of

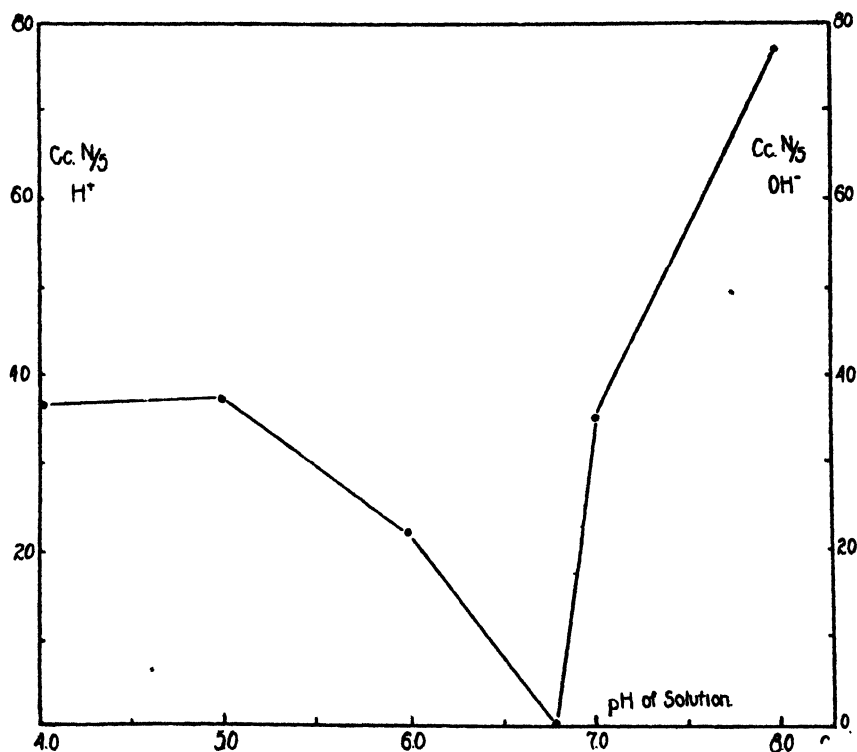


Fig. 5

growth is represented graphically for each type. These curves are not strictly comparable, since the plants were grown at different times of the year. The amounts of acid or alkali neutralized within a definite period of time depend largely upon the rapidity of growth.

Although the curves are only of a qualitative significance, they are very expressive of the power of the plant to overcome any unfavorable acidity or alkalinity, especially the latter. This power is of obvious importance to the plant and must form an integral part of any study of acid or alkali resistant crops, either in the soil or in

solution culture. Under natural conditions, the plant has to contend with the reaction of the medium in which its roots are immersed or imbedded from the time of germination to maturity. If the medium is sufficiently highly buffered or is continually renewed, such that little or no change of reaction is brought about under the influence

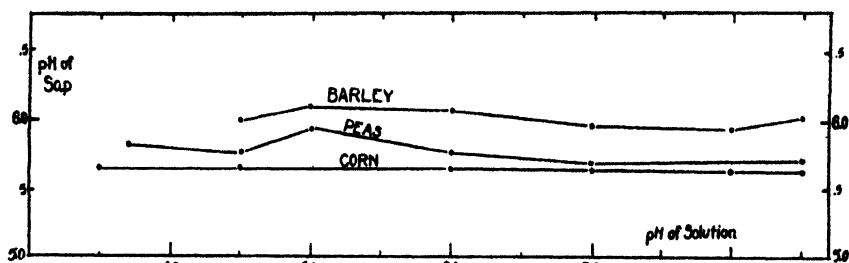


Fig. 6

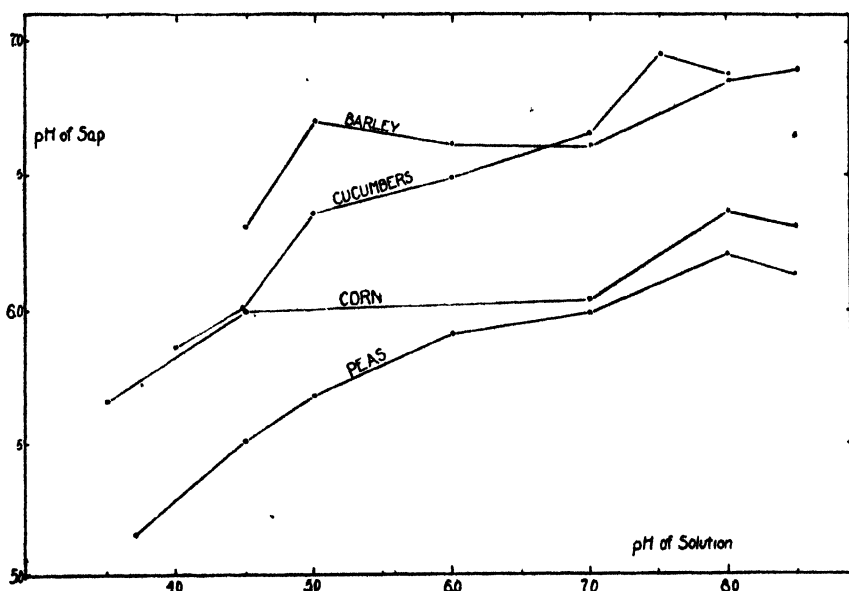


Fig. 7

of the plant, the ability to overcome any unfavorable reaction is correctly expressed by these curves. From a purely theoretical point of view, however, this ability may be determined at different reactions for plants treated similarly up to the time of experimentation, so that the vigor and internal mechanism of all the plants will as nearly

as possible be the same when subjected to the different acidities or alkalinities. These must be such that the plant mechanism will not be injured or altered materially during the period of experimentation.

Five sets of 25 barley plants each were grown in earthenware crocks of  $7\frac{1}{2}$  liter capacity. All the solutions had a reaction of  $P_H$  6.8, and were changed weekly. When plants were four weeks advanced, the sets were transferred to solutions having the reactions 4.0, 5.0, 6.0, 7.0, and 8.0, and these were kept as constant as possible for four days by the addition of N/5 acid or alkali. It was assumed that the sets of plants were not affected materially by the differences in reactions within this period of time.

In figure 5, the amounts of N/5 acid or alkali neutralized are plotted against the desired  $P_H$  as before. Unfortunately the number of determinations made are insufficient to permit of the smoothing out of the curves. Their general shape, however, is obvious. On the alkaline side of  $P_H$  6.8, the ability to neutralize excessive concentrations of hydroxyl ions increases very rapidly with the increase in  $P_H$  and probably does not reach a maximum even at  $P_H$  8.0. On the acid side, however, the increase is less rapid and reaches a maximum between  $P_H$  4.0 and 5.0.

#### INFLUENCE OF FACTORS OTHER THAN THE REACTION

The plants were grown in the open during the summer months and in a heated greenhouse during winter. In the course of the investigation, it became evident that plants grown at different seasons show slight differences in their resistance to the effect of the reaction. This is most probably due to the differences in the rate of growth under different atmospheric conditions.

The influence of the composition of the culture solution on the effect of the reaction was not determined, as only one solution was used throughout the investigation. It is highly improbable, however, that the composition of the solution, within wide limits, is a factor in any of the divers phases of this study. The results obtained by Salter and McIlvaine<sup>27</sup> and those obtained by the writer seem to substantiate this assumption.

The amounts of water transpired by plants from solutions of different reactions were found to be the same within the limits of the experimental error.

## DISCUSSION

The conclusion reached by earlier workers<sup>2, 4, 22</sup> was that the H ion was more toxic than the OH ion to plants growing in solution cultures. Their results, however, are untenable because they failed to distinguish between potential and actual acidity or alkalinity. The ability of the plant to change the reaction of the nutrient medium was likewise overlooked.

In a series of papers, Hoagland<sup>15, 16, 17, 18</sup> has called attention to both these factors and showed that the OH ion is much more toxic to barley seedlings in solution culture than the H ion. An OH ion concentration greater than  $P_H$  8.2 was distinctly injurious, whereas an H ion concentration of  $P_H$  5.0 was found to be favorable to growth and to cause no injury. Similar results were obtained by Duggar<sup>5</sup> using various types of solutions and growing the plants under the most diverse environmental conditions. One of the most complete and satisfactory studies on this problem is that of Salter and McIlvaine.<sup>27</sup> These investigators experimented with corn, wheat, soybeans, and alfalfa, growing the plants at seven different H ion concentrations. The plants were grown for relatively short periods of time and the solution changed once every four days. A distinct maximum in the growth of the plants was found at  $P_H$  5– $P_H$  6. At a neutral reaction, decided decreases in the yields could be demonstrated.

We have already called attention to the advisability of growing the plants for a considerable length of time so as to overcome the influence of the food supply stored in the seed. Only in this way is it possible to obtain a true measure of the effect of the reaction of the solution. The growth periods employed by these investigators were undoubtedly too short. On the other hand, the variations in reaction caused by young plants are relatively small, so that plants grown in accordance with the technique they employed will give more reliable results if the experiment is discontinued after two weeks than if the plants are grown for a longer period of time. Our results agree substantially with those of these investigators.

Hixon<sup>14</sup> found a distinct minimum in the development of young plants as measured by the growth in length of the roots and tops at  $P_H$  5 for *Pisum* and  $P_H$  6 for most other plants. This minimum point is interpreted as that of greatest efficiency and the point of normal growth. We have been able to confirm his results in part. A decided stimulation occurred at acidities which injured the plants definitely

later on. No stimulation was noticed in the tops of plants grown in alkaline solutions. The roots were occasionally longer than those of the plants grown at  $P_H$  5 or  $P_H$  6.

A glance at figures 4 and 5 is sufficient to make evident the importance of controlling the reaction of the solution under investigation.

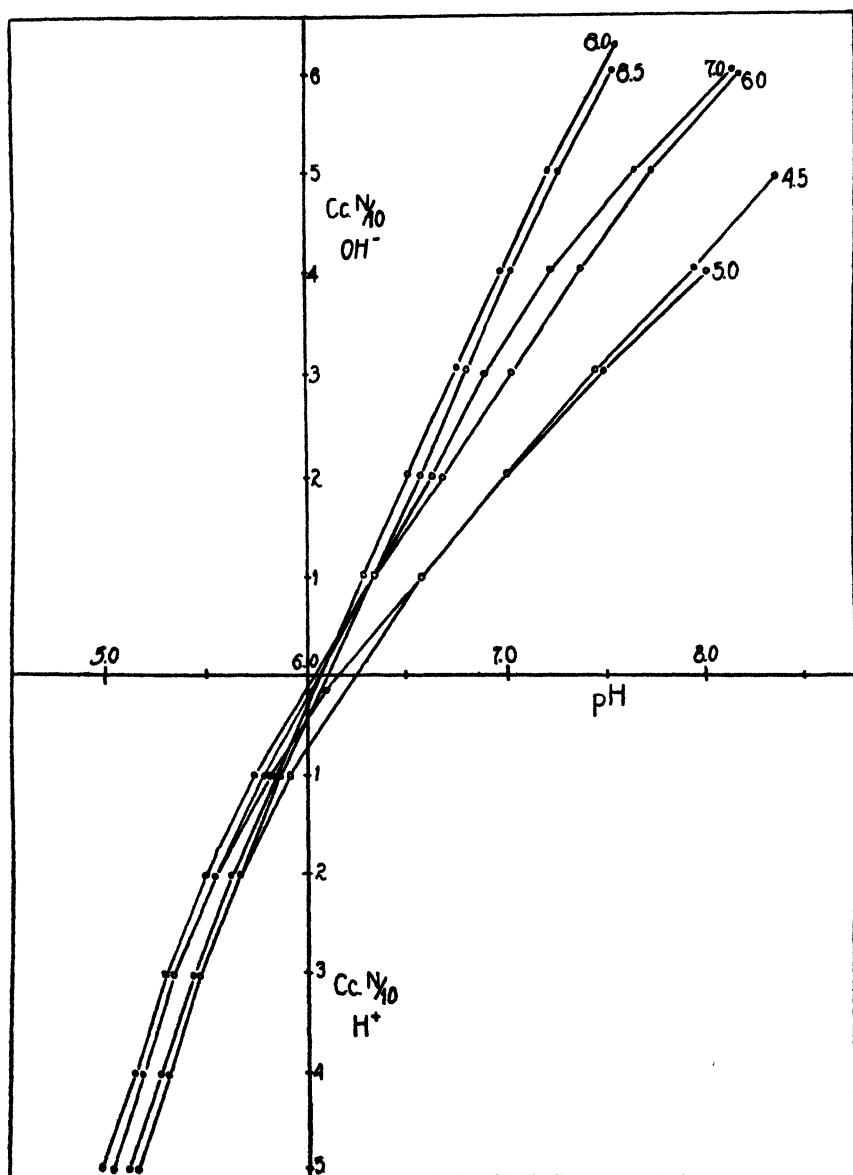


Fig. 8



Changes of solution every fourth or fifth day are obviously insufficient to maintain the  $P_H$  constant even approximately, when the plants are three to four weeks advanced. With small volumes of solutions supporting relatively large numbers of plants grown at  $P_H$  5, this becomes increasingly difficult. In an investigation by McCall and Haag,<sup>23</sup> this point seems to be lost sight of completely. From their investigations, it appears that wheat plants grow best at reactions between  $P_H$  3 and  $P_H$  4. It is very plain, however, that the reactions of the solutions in the neighborhood of the roots must have been very different from what they were assumed to be. It is not strange that the solution with the highest buffer effect gave the poorest growth.

In culture solutions, the diffusion of solutes is relatively rapid and as a rule the reaction around the roots is the same as that in the bulk of the solution. If, however, the free diffusion is interfered with, such as often happens among the roots in the upper few inches of the solution, the reaction may be very different in this region from what it is in the bulk of the solution. (Over the ranges of low buffer effect, a difference of 0.5  $P_H$  can occasionally be demonstrated under such conditions. In soils, the diffusion is infinitely slower and the reaction of the solution in contact with the absorbing roots will be determined solely by the ability of the plant to overcome the buffer effect of the soil complex in its immediate vicinity. Considering the power of growing plants to regulate the  $P_H$  value of the culture medium, the conclusion is inevitable that the direct effect of the actual reaction of most soils can hardly be a factor in the complex which determines the growth of the plant in that soil, provided the plant has the ability to establish itself firmly. In this connection the work of Joffe<sup>19</sup> with alfalfa is very elucidating. The results obtained with solution cultures agree well with those of this investigator using soils acidified artificially.

From the determination of the reactions of numerous acid soils reported by Gillespie,<sup>9</sup> and Sharp and Hoagland,<sup>28</sup> it is apparent that the reaction of the majority of these soils can have little or no direct effect on the growth of plants. The infertility of acid soils can usually be ascribed to causes other than the H ion concentration. The solubility of aluminum in the slightly acid soil solutions of these soils is undoubtedly responsible for some of the phenomena attributed formerly to the acidity of the soil.<sup>1, 12, 24, 25, 29</sup>

The soil solution of many alkali soils has a highly alkaline reaction, which tends to prevent the young plants from germinating or develop-

ing. Germinating seeds have a remarkable ability to change the reaction of the alkaline medium in which they are immersed, in the direction of neutrality, so that the  $P_H$  value around the seeds may be made favorable to germination. The ability of the seedling to

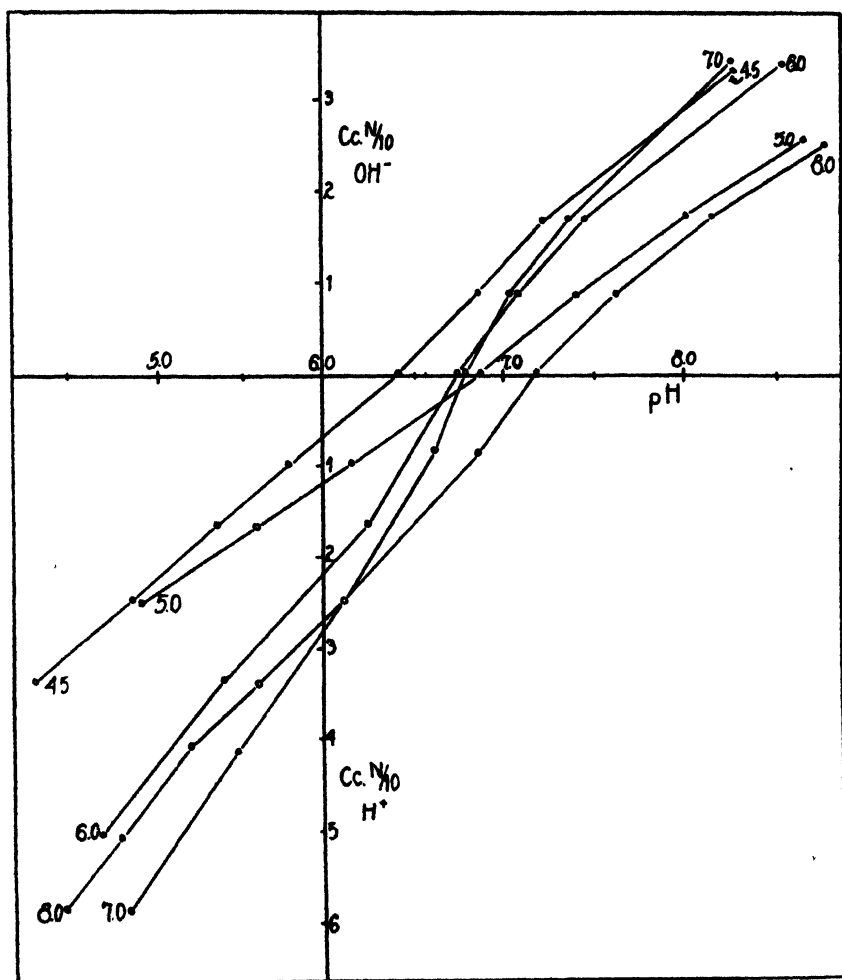


Fig. 9

regulate the reaction is comparatively small and hence the young roots may be unable to penetrate beyond the regions of the favorable reaction brought about by the seed. If the soil solution has both a high  $P_H$  value and a high concentration of salts, the seedlings will naturally be unable to survive.

## EFFECT OF REACTION OF CULTURE SOLUTION ON THE REACTION AND BUFFER EFFECT OF THE PLANT JUICES

The plants from the experiments described above were frozen immediately after they were harvested. This was done in a cold room kept at 12° F., from which they were only removed as they were needed. The plant juices were obtained by grinding the frozen mass, thawing this rapidly in a warm room, and then expressing the sap by hand through a few thicknesses of cheesecloth. All determinations were made as soon as possible after the frozen ground material was thawed out.

### THE H-ION CONCENTRATION OF THE SAP

The reaction of the juices of the roots and tops, obtained in the above way, was measured by means of a Hildebrand hydrogen electrode. Difficulty was experienced in making the measurements as reduction of  $\text{NO}_3$  ions apparently took place on the electrode. This was especially true in the case of the juices from those plants grown at the acid reactions. This difficulty was obviated to some extent by leaving the  $\text{NaNO}_3$  out of the culture solution during the last week of the experiments.

In figures 6 and 7, the reactions of the tops and roots of cucumber, barley, pea, and corn plants grown at different reactions are represented graphically. The reactions of the sap expressed from the tops were not influenced by the reaction of the culture solution, the variations in reaction being within the limits of the experimental error.

On the other hand, the reactions of the root juices are decidedly changed by the reaction of the solution.\* It is plain, however, that the reactions of the roots are very different from the reactions of the solutions, except when these are between  $P_H$  6 and  $P_H$  7. Whether the reaction of the root juices is influenced according to any definite rule by the reaction of the solution, as may be suggested by the curve for pea roots, it is impossible to say at present, owing to the relatively large experimental error involved in these measurements. Truog and Meacham,<sup>31</sup> after studying the effect of additions of lime to a soil, concluded that the reaction of the soil can influence the reaction of the sap expressed from the tops of the plants. It seems obvious, however, that the differences in the reactions from the limed and unlimed plots

\* Compare Bryan, O. C., Effect of different reactions on the growth and nodule formation of Soy beans. *Soil Science*, vol. 12, no. 4, pp. 271-287 (1922).

are within the experimental error, apart from the fact that many other factors enter in the case of plants growing in limed and unlimed acid soils.

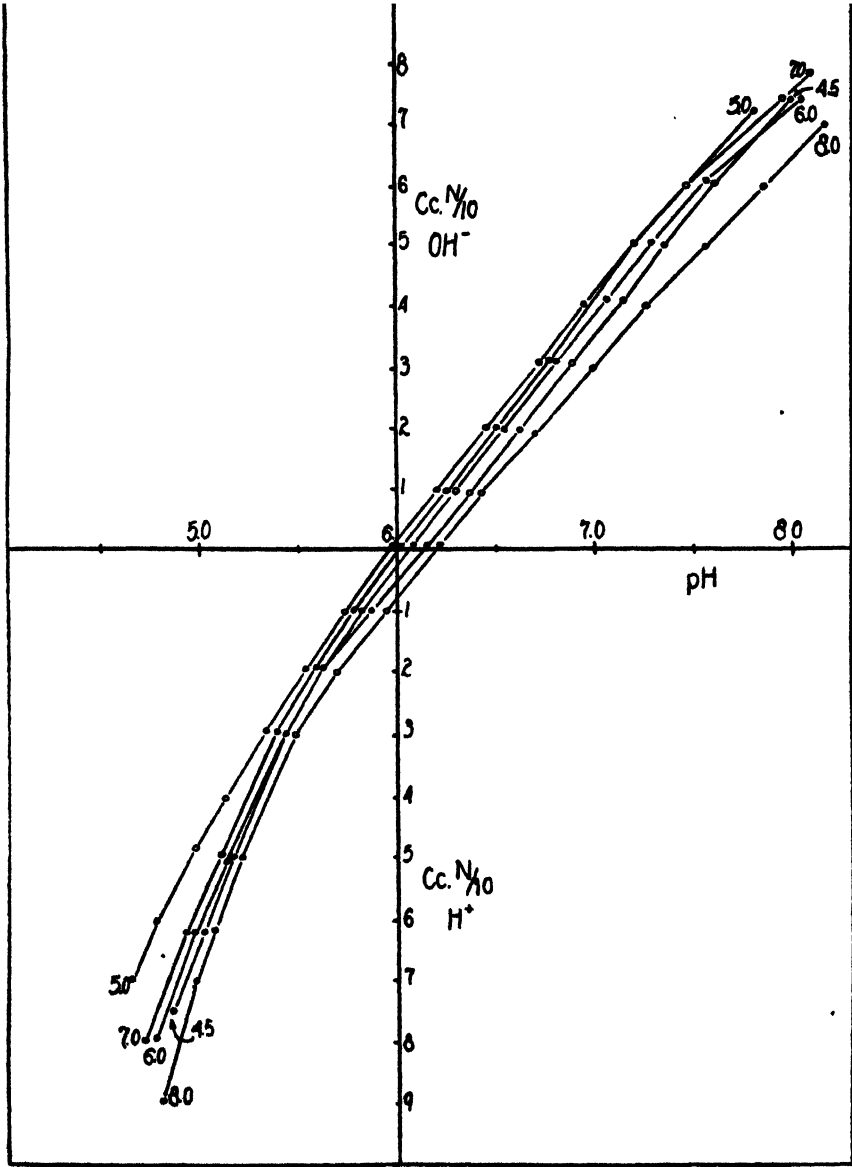


Fig. 10

## THE BUFFER EFFECT OF THE SAP

The juice obtained from the plants in the way described was titrated electrometrically (after an equal volume of water had been added) with N/20 acid and alkali. The  $P_H$  value was invariably increased by about one-tenth of a magnitude of the dilution.

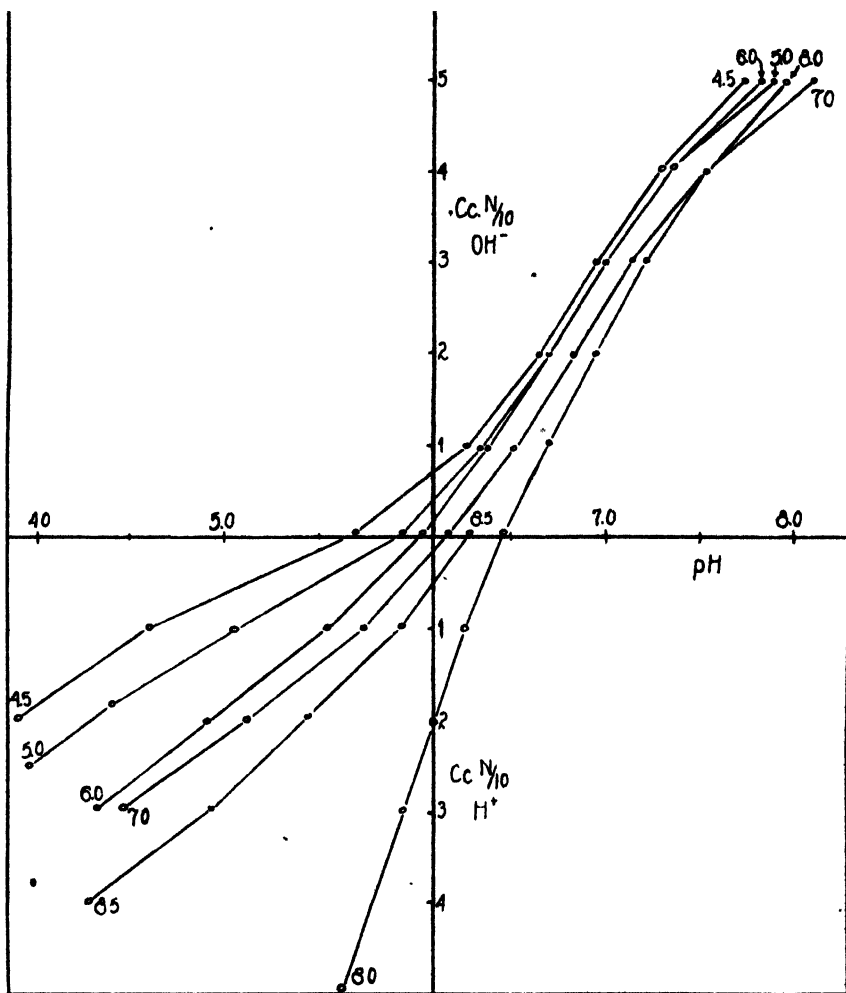


Fig. 11

In figures 8 and 9 the titration curves for barley tops and roots, respectively, are given. The curves are represented as if 25 c.c. of undiluted sap had been titrated with N/10 reagents. The corresponding curves for peas are given in figures 10 and 11.

Hempel<sup>18</sup> has shown that the buffer effect of plant juices is mainly due to the organic acids and salts of these acids contained in the plant system. It appears from figure 8 that the reaction of the nutrient solution has influenced the concentration of those acids with dissociation constants less than  $10^{-6}$  very markedly in the tops of barley plants although the reaction of the expressed sap is apparently unchanged.

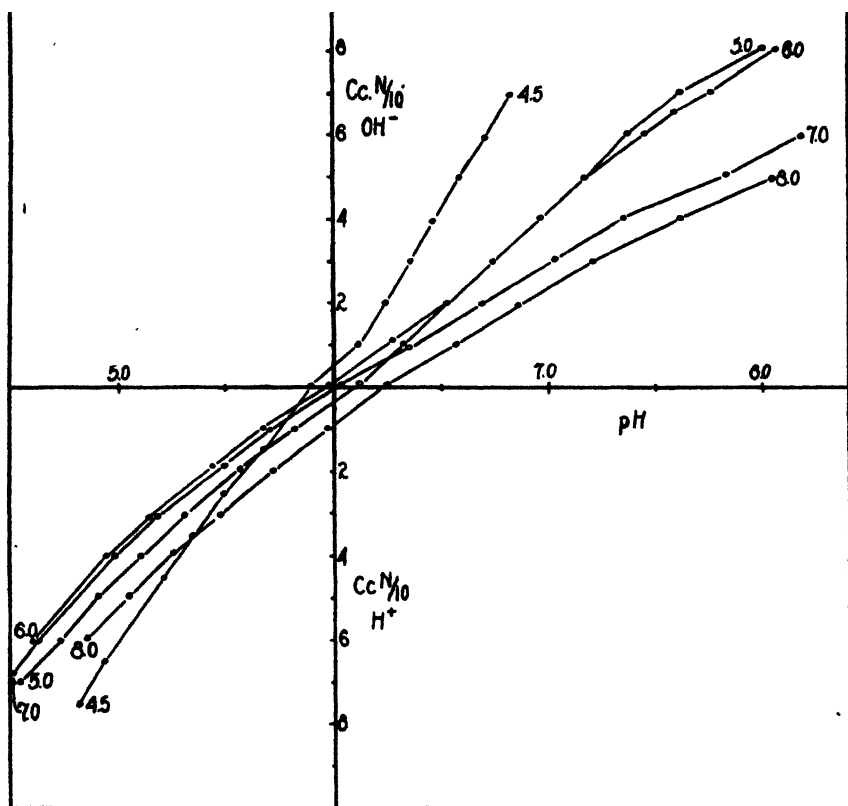


Fig. 12

In the roots the buffer effect is also influenced. Here, however, only those acids with a dissociation constant higher than  $10^{-6}$  are affected. In the case of the pea plants, neither the reaction nor the buffer effect of the sap expressed from the tops was influenced by the reaction of the nutrient medium. The roots on the other hand were affected similarly to the barley roots. These plants were grown in a greenhouse during the winter.

In figure 12, the results of a similar experiment with peas grown in the open in summer are given. In this experiment, the reaction of

the sap expressed from the tops was unchanged, but the buffer effect was influenced by the reaction of the culture solution. The effect, however, is the reverse of what it was in the case of the tops of the barley plants, and in both instances was only noticeable in the concentration of those acids with a dissociation constant lower than  $10^{-6}$ .

Unfortunately, it was not possible to pursue this line of investigation with additional plants and under the different atmospheric conditions. It seems, however, that a thorough study along these lines will throw considerable light on the salt metabolism of plants.

### SUMMARY

1. The influence of the reaction of the culture medium on the growth and metabolism of the common agricultural plants was studied by growing typical plants in solution cultures at different reactions.

2. After experimenting with several different methods, a technique was devised by which the reaction of the solution could be conveniently controlled. Particular attention was given to the constant maintenance of the desired hydrogen-ion concentration during the experimental periods.

3. Plants grown in solution cultures have an optimum growth reaction at  $P_H$  4.5 to  $P_H$  6.

4. The reaction of the juice expressed from the tops of the plants was not influenced by the reaction of the culture medium, whereas the reaction of the juices expressed from the roots was modified considerably.

5. The buffer effect of both the roots and the tops may be influenced by the reaction of the culture solution. In the tops, the acid reserve is affected and in the roots, the alkali reserve.

6. Observations were made on the ability of the growing plant to change the reaction of either acid or alkaline culture solutions.

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## II. POSSIBLE MECHANISM OF THE PLANT'S INFLUENCE ON THE REACTION OF THE CULTURE SOLUTION

Recent studies on the absorption of inorganic ions by plants as well as the large amount of work done on the problem of the antagonism between ions and the physiological balance in culture solutions have thrown some light on the mechanism by which the plant obtains its inorganic elements. The importance of a more thorough knowledge of this process is undisputed. Unfortunately investigations on this problem are hampered by our meager knowledge of the true nature of solutions and the methods of analysis at our disposal.

In considering the absorption of any ion, account must be taken of the activities of that ion inside and outside of the membrane effective in absorption. We are at present unable to determine the activity of any ion in a system as complex as a complete culture solution except that of the H ion, which can usually be determined with sufficient accuracy.

Since the activity and the total molal concentration of the H ion are conveniently and rapidly determined and since we have every reason to believe that the H and OH ions are absorbed, fundamentally, in the same way as any other positive or negative ion, we have here a very efficient means of studying this problem.

In Part I of this investigation, a series of experiments were described which were concerned mainly with the effect of the reaction of a culture solution on the growth and metabolism of several types of plants. In the present paper, some preliminary experiments are described which are concerned with the effect of the growing plant on the composition and especially on the reaction of culture solutions.

Pantanelli<sup>3</sup> observed that plants always changed the reaction of a single salt solution in the direction of neutrality except when  $(\text{NH}_4)_2\text{SO}_4$  was the solute. In a solution of this salt, the reaction remained at the initial value, namely  $P_H$  5. Similar results were obtained by Hoagland<sup>4</sup> with barley plants. Later work shows that solutions of  $(\text{NH}_4)\text{Cl}$ ,  $\text{K}_2\text{SO}_4$  and some other salts behave similarly to  $(\text{NH}_4)_2\text{SO}_4$ . The reaction may even change appreciably toward a higher acidity especially when the plants have not been previously grown in a complete culture solution. When complete culture solutions were used, the reaction was invariably changed toward neutral-

ity. These results with complete culture solutions were confirmed by Duggar<sup>2</sup> and several later workers for different types of plants and solutions. Jones and Shive<sup>5</sup> found that the reactions of 20 representative solutions of the Tottingham series were changed toward neutrality. When, however,  $(\text{NH}_4)_2\text{SO}_4$  was substituted for  $\text{KNO}_3$  in these solutions, the reactions remained practically constant at the original value, namely  $\text{P}_\text{H}$  4.8.

In the present investigation, all the plants experimented with invariably changed the reaction of the complete culture solution toward some point between  $\text{P}_\text{H}$  6.5 and  $\text{P}_\text{H}$  6.9, irrespective of what the original concentration might have been (see Part I).

The exact mechanism by which the plant changes the reaction of a solution has not been established. In a uni-salt solution, this may be ascribed to ionic exchanges, and on the alkaline side, the OH ions are partly neutralized by carbon dioxide excreted from the roots. In a complete culture solution, the problem becomes more complex. It will thus be of advantage to tabulate the different methods which a growing plant conceivably might have at its disposal for changing the reaction of the solution.

The decrease of H ion concentration would be accomplished:

1. By neutralizing H ions by OH ions derived from some base excreted by the roots or from dead root cells.
2. By absorbing H ions and simultaneously replacing these by some other positive ion.
3. By absorbing an anion and excreting OH ions simultaneously.
4. By absorbing H ions and an equivalent amount of some negative ion.
5. By absorbing an anion and excreting simultaneously another anion which forms an acid with a lower degree of dissociation or an acid which is volatile under the conditions.

The H ion concentration is increased:

- (a) If OH ions are neutralized by H ions derived from some acid excreted by the plant or from dead root cells.
- (b) If OH ions are absorbed but simultaneously replaced by some other anion.
- (c) If OH ions and an equivalent amount of a cation are absorbed simultaneously.
- (d) If a positive ion is absorbed and replaced by H ions.

Excretions by the roots are confined to the acid  $\text{HCO}_3^-$  (or  $\text{CO}_2$ ) and, under certain conditions, small amount of cations, notably

calcium. The quantities of the latter are, however, insufficient to account for more than a very small part of the power of the plant to change the reaction of the solution. The increase in the  $P_H$  value must thus be accounted for by methods 3, 4, or 5.

The decrease in the  $P_H$  value of a culture solution might take place by any or all of the methods outlined under *a*, *b*, *c*, and *d*. In some cases the  $P_H$  value of the solution is decreased to about  $P_H$  3.2, as frequently happens in a uni-salt solution of  $K_2SO_4$ , for example. Since the concentration of the OH ions is very small at this reaction, it is possible that method *d* is chiefly involved. Method *c*, however, cannot be excluded from consideration.

The plant has a very efficient means at its disposal for reducing the alkalinity of a solution in that it normally excretes relatively large amounts of  $CO_2$  (method *a*). This, however, is not the only mechanism involved as is apparent from the results of the following experiment. Corn plants growing in a complete culture solution maintained at  $P_H$  8.5, as was described in Part I, neutralized within one week 0.0257 equivalents of alkali. When the solution was analyzed only 0.0185 equivalents of  $CO_2$  were found. Hence approximately one-fifth of the alkali added must have been neutralized by methods *c* and *d*. Whether this neutralization was brought about with either or both of these methods, the final composition of the solution would be the same. The solution must have lost approximately 0.007 equivalents of cations, except H-ion and an equivalent amount of OH ions.

The conclusion, then, is inevitable that, exclusive of the H and OH ions, greater equivalent proportions of anions than of cations must be absorbed on the acid side. On the alkaline side, the reverse must be true.

The rate of absorption of either the anions or the cations, or both, may be influenced by differences in the reaction of the culture medium in order to bring about this selective absorption.

Several experiments were carried out to obtain some preliminary information on this point. The problem was attacked by means of absorption studies, the relative amounts of the different ions absorbed at different reactions by similar plants being determined. For the purpose of these experiments, actively growing four-week-old plants were used. The plants were grown in earthenware crocks, the solution used being identical with the culture solution described in Part I, except that  $KNO_3$  was substituted for  $NaNO_3$ . The reactions were maintained at certain definite values by means of the technique

described in Part I, and the absorption was allowed to take place over a period of from 3 to 4 days, after which the solutions were made up to the original volume and analyzed.

The results of experiments with barley and cucumber plants are given in tables 1 and 2, respectively. Similar experiments were carried out with peas.

TABLE 1

ABSORPTION OF ANIONS AND CATIONS BY BARLEY PLANTS AT DIFFERENT REACTIONS

Period of absorption	No. of plants	PH at which solution was maintained	Total weights absorbed, gms.				Mg
			NO <sub>3</sub>	PO <sub>4</sub>	K	Ca	
3 days	30	4.5	.7144	.1824	.222	— .018	.023
3 days	30	8.0	.5952	.1856	.293	.006	.038

TABLE 2

ABSORPTION OF ANIONS AND CATIONS BY CUCUMBER PLANTS AT DIFFERENT REACTIONS

Period of absorption	No. of plants	PH at which solution was maintained	Total weights absorbed, gms.			
			NO <sub>3</sub>	PO <sub>4</sub>	K	Ca
3 days	25	5.0	.2557	.0646	.060	— .0010
3 days	25	6.0	.2425	.0420	.105	.0101
3 days	25	7.0	.2020	.0720	.180	.0169

It is apparent that the influence of the reaction is most marked on the rate of the absorption of the cations. Invariably there were more cations absorbed from the alkaline than from the acid solutions, i.e., the rate of absorption of cations was increased by a decrease in the concentration or activity of the H ion and vice versa. This implies a relative increase of the activity of the cations in the solutions over that in the plant. It is more probable that the activity of the cations in the plant is decreased than that the activity of the cations in the solution is increased so as to bring about such a marked change in the rate of absorption of the cations.

Loeb's<sup>6</sup> brilliant researches have thrown much light on the relation existing between inorganic salts or ions and charged organic colloids and the distribution of ions on the two sides of a membrane when one side contains an ion which cannot diffuse through the membrane. To what extent the principles discovered by him may apply to the absorption of salts by plants it is impossible to say at present. If it be assumed that ionic equilibria are established between the roots

and the solution, these principles will undoubtedly determine the equilibrium concentration of the ions. Unfortunately, however, the existence of such equilibria in plant cells has not been established definitely. Assuming, however, that an equilibrium is established between the ions in the cells, which are active in absorption, and the ions in the solution, the effect of the reaction on the rate of absorption of the cations is readily explained as will be apparent from the following considerations.\*

In the cell there are, among other substances, anions of organic acids, salts of these acids and probably of free acids, to which the membranes effective in the absorption are impermeable, and also complex colloidal bodies which are probably negatively charged (see below). Since the reaction of the cells of the roots (in so far as it is reflected in the plant juices) is influenced markedly by the reaction of the solution (see Part I), it is clear that an acid reaction of the culture solution will have the effect of depressing the dissociation of these acids and negatively charged bodies, and consequently the number of cations held by electrostatic forces will be decreased, i.e., the activity of the cations will be increased. In other words, the rate of absorption of the cations will be decreased by an increase in the H ion concentration. As a rule, a greater number of equivalents of nitrate ions were absorbed on the acid than on the alkaline side, whereas the absorption of phosphate ions was very irregular. Unfortunately, the ease with which a plant is able to replace certain anions absorbed by  $\text{HCO}_3$  ions complicates attempts to determine whether or not the rate of absorption of anions is affected by the reaction of the culture solution. If the root contains any positively charged bodies to which the membranes are not permeable, we would expect an increase in the H ion concentration to produce an increased rate of absorption of the anions.

The charge on the proteins and other amphoteric bodies which constitute protoplasm becomes of paramount importance in this connection. To gain some information on this point resort was made to cataphoresis experiments. A slightly modified form of the apparatus described by Cohn, Gross, and Johnson<sup>1</sup> was used for the purpose,

\* Since this paper was completed, work has been reported by the Laboratory of Plant Physiology of Harvard University and by the Laboratory of Plant Nutrition of the University of California, which indicates that additional considerations must be taken into account. For example, experiments on the alga *Nitella* (from which uncontaminated cell sap may be obtained) prove that an ion may be absorbed from a solution of low concentration into a solution of high concentration, and that certain inorganic elements, such as potassium, exist in the cell almost entirely in ionic form.

and the migration of the nitrogenous constituents in the sap, expressed from the roots after freezing, was determined by analyzing the buffer mixtures in the cathodic and anodic chambers for nitrogen by the Kjeldahl method. In the case of the juices from the roots of barley, pea, and cucumber plants, the migration was invariably found to be toward the anode, proving that these bodies are charged negatively at the reaction at which they occur in the plant. The direction of migration was not reversed at a reaction of  $P_H$  4.5. The same results were found when the root juices were well dialysed against distilled water. Since the reaction of the juice, when it is freshly expressed from the tissue, has a reaction of approximately  $P_H$  6, it is plain that the isoelectric points of the nitrogenous bodies are considerably below the  $P_H$  values at which they normally occur in the sap.

Deductions drawn from experiments with the expressed sap can hardly be considered as applying to the living root, which is a highly differentiated structure. The process of freezing may bring about changes sufficiently severe to change the sign of the charge on some of the ampholytes in the living cell or to cause mutual precipitation of oppositely charged colloids from the same or from different cells. In general it is improbable, however, that the isoelectric points of the different ampholytes will be changed materially by this treatment. Since the former are so far removed from the reaction at which these ampholytes occur in the root tissues, it is highly probable that the majority of the proteins and other nitrogenous bodies are charged negatively in the living cell, and that the sign of the charge is not readily reversible as assumed by Haynes.<sup>8</sup> The work of Meier<sup>7</sup> substantiates the above conclusions. This investigator found that the cell contents in the roots of actively growing plants moved under the influence of a small current as if they were negatively charged.

If a plant be allowed to change the reaction of an acid or alkaline solution, a certain  $P_H$  must be reached at which the tendency of methods 3, 4, and 5 to decrease the H ion concentration is balanced by the tendency of methods *a*, *c*, and *d* to decrease the OH ion concentration. Because of the many factors involved in this equilibrium, one can hardly expect this reaction to be very definite under the varying conditions of experimentation. For barley and corn, this value was found to be  $P_H$  6.75 to  $P_H$  6.8, and for peas,  $P_H$  6.65 to  $P_H$  6.7.

The significance of this point is not known at present. The main factor involved in bringing about this reaction in a solution seems to

be the activities of the  $\text{CO}_2$ ,  $\text{H}_2\text{CO}_3$ , and the ions of this acid in the plant and in the solution. If this equilibrium is disturbed in such a way as to allow the escape of  $\text{CO}_2$ , as may happen when the volume of the solution is diminished excessively by transpiration, the  $\text{P}_\text{H}$  value must rise. This is easily demonstrated by allowing the solutions in the containers to 'run down.' The reaction may rise to as high as  $\text{P}_\text{H}$  8.5. On the other hand, if the other factors which contribute toward this equilibrium be missing, the  $\text{P}_\text{H}$  value will decrease till an equilibrium is established between the  $\text{CO}_2$  of the atmosphere above the solution and the  $\text{H}_2\text{CO}_3$  and  $\text{HCO}_3^-$  in the solution. Such a condition is brought about in distilled water in which the reaction is maintained at a slight acidity. The equilibrium reaction also depends upon the rapidity with which the different ions are absorbed. In solutions in which the rapidly absorbed anion  $\text{NO}_3^-$  is replaced by the rapidly absorbed cation  $\text{NH}_4^+$ , as in the investigation by Jones and Shive,<sup>5</sup> the equilibrium will naturally be thrown over to the acid side.

From the above considerations, it is obvious that an 'optimum' culture solution for the growth of plants will depend not only on the composition of the culture solution, but also upon the partial pressure of the  $\text{CO}_2$  in the atmosphere and other atmospheric conditions.

The fact that the optimum reaction for the growth of plants in solution culture is on the acid side is possibly correlated in part with the greater ease with which the respiratory  $\text{CO}_2$  can diffuse out and away from the roots at this reaction. If this theory is correct, the optimum reaction will even be slightly more toward the acid side in soil, since the diffusion of  $\text{CO}_2$  is interfered with.

## SUMMARY

1. A study of the effect of the reaction of the solution on the absorption of the anions and cations by the plant is described.
2. Several methods are outlined by which the plant changes the reaction of either acid or alkaline culture solutions toward neutrality.
3. Absorption experiments show that the rate of absorption of the cations is increased by a decrease in the H-ion concentration, while the ability of the plant to excrete  $\text{CO}_2$  from the roots allows of the selective absorption of anions from the acid solutions.
4. The charge on the constituents of the root cells may be assumed to be of vital importance in the mechanism of absorption.



5. The nitrogenous constituents of the cell sap are charged negatively and the isoelectric points of the majority of ampholytes in the cell is below  $P_H$  4.5.

6. Pea plants change the reaction of either acid or alkaline solutions from  $P_H$  6.65 to  $P_H$  6.7, whereas barley and corn plants change it from  $P_H$  6.75 to  $P_H$  6.8. The main factor involved in bringing about this reaction is the  $CO_2$ - $HCO_3^-$  equilibrium in the plant and in the solution.

*Transmitted, March 23, 1923.*

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